*Volume 33, Number 4, February, 1961*

# The Yale Journal of Biology & Medicine


259 **REBOUND IN ACUTE RHEUMATIC FEVER**
**I. INCIDENCE AND SIGNIFICANCE** *A. R. Feinstein, M. Spagnuolo, and F. A. Gill*

279 **II. TREATMENT AND PREVENTION** *M. Spagnuolo and A. R. Feinstein*

299 **ADENOHYPOPHYSIS IN NEOPLASTIC DISEASE** *A. Golden, P. K. Bondy, R. W. Chambers, and C. L. Geiger*

308 **HEPATIC LIPID AND GOLDTHIOGLUCOSE-OBESITY** *J. S. Meyer, H. Imai, and H. W. Scott*

318 **EXPERIMENTAL PYELONEPHRITIS** *L. R. Freedman and P. B. Beeson*

333 **BOOK REVIEWS**

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## CONTENTS


259 **THE REBOUND PHENOMENON IN ACUTE RHEUMATIC FEVER**
**I. INCIDENCE AND SIGNIFICANCE** *Alvan R. Feinstein, Mario Spagnuolo, and Fred A. Gill*

279 **II. TREATMENT AND PREVENTION** *Mario Spagnuolo and Alvan R. Feinstein*

299 **ADENOHYPOPHYSEAL CHANGES IN PATIENTS DYING OF NEOPLASTIC DISEASE** *Abner Golden, Philip K. Bondy, Robert W. Chambers, and C. Leonard Geiger*

308 **HEPATIC LIPID IN GOLDTHIOGLUCOSE-OBESE MICE** *J. S. Meyer, H. Imai, and H. W. Scott*

318 **EXPERIMENTAL PYELONEPHRITIS**
**VIII. THE EFFECT OF ACIDIFYING AGENTS ON SUSCEPTIBILITY TO INFECTION** *Lawrence R. Freedman and Paul B. Beeson*

333 **BOOK REVIEWS**

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*Subscriptions.* THE YALE JOURNAL OF BIOLOGY AND MEDICINE is published in August, October, December, February, April, and June from 333 Cedar Street, New Haven 11, Connecticut, U.S.A. The subscription price for one volume of six issues is $7.50 in the United States and other countries. For students registered in any school of Yale University, the subscription price is $4.00 per volume. Single copies are $1.50 each except for special issues.

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*Subscription rates:* $10.00 per annum; single copies $3.00.

PUBLISHED QUARTERLY FROM THE DEPARTMENT OF THE HISTORY OF SCIENCE AND MEDICINE, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

*Distributor in Great Britain:* Lloyd-Luke (Medical Books) Ltd., 49 Newman Street, London, W.1.

ALVAN R. FEINSTEIN*
MARIO SPAGNUOLO**
FRED A. GILL†

*Irvington House, Irvington-on-Hudson, New York, and the Department of Medicine, New York University School of Medicine, New York*

# THE REBOUND PHENOMENON IN ACUTE RHEUMATIC FEVER
## I. INCIDENCE AND SIGNIFICANCE‡

When anti-inflammatory treatment is reduced or stopped in patients with acute rheumatic fever, clinical or laboratory evidence of rheumatic activity sometimes reappears. This phenomenon had occasionally occurred after salicylate therapy[9, 13] in the pre-steroid era, but the name "rebound" came into use only in recent years[6, 22] when the incidence of the event was so frequent after steroid treatment that a specific title was given to it.

Studies of the rebound phenomenon are sparse[5, 6, 7, 10, 11, 22] and have been performed under two sets of disadvantages. One difficulty has been the selection of patients and therapy:

1. The number of patients has been small[6, 7, 10, 22] or chosen from an adult population.[5, 11]
2. Since the duration of drug therapy in several studies[7, 11, 15] was identical for all patients, the effects of different *lengths* of treatment could not be determined.
3. The post-therapeutic follow-up period was often short so that the occurrence of late rebounds could not be detected.

A second difficulty has been the identification of the phenomenon:

1. The "rebound" is not uniformly defined, and is described by varying criteria.
2. Rebounds where the only abnormalities were in laboratory tests, have not often been distinguished from those which included clinical features; in the latter group, cardiac and noncardiac events have not been separated.
3. The pre-therapeutic clinical status of the patients has seldom been correlated with the features of the associated rebound.

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* Medical Director, Irvington House; Assistant Professor of Medicine, New York University School of Medicine.
** Senior Research Fellow, Irvington House.
† Senior Medical Student, Northwestern University School of Medicine.
‡ Supported in part by fellowships from the New York State Department of Health and by the National Science Foundation.
*Received for publication 4 June 1960.*

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4. Bacteriological and immunological techniques have rarely been used to demonstrate the absence of intercurrent streptococcal infections, in order to prove that the recurrent inflammation was a post-therapeutic rebound rather than a new attack of rheumatic fever.

As a result of these inconsistencies, the rheumatic-fever rebound phenomenon has been poorly understood and has received various interpretations. It has been attributed to (*i*) polycyclic rheumatic fever ,(*ii*) withdrawal of treatment before the natural course of inflammation had subsided, (*iii*) relative adrenal insufficiency, or (*iv*) the accumulated rheumatic inflammation whose clinical outlet had been prevented by the preceding suppressive treatment. The relative importance of these four possible causes is uncertain.

The present survey has studied the incidence and significance of the rebound phenomenon in the attacks of acute rheumatic fever in 265 children and adolescents, treated with a variety of agents and followed throughout the convalescent period and beyond. The results help clarify the nature of the rebound process and have clinical and conceptual implications for the natural history and management of rheumatic activity.

## CLINICAL MATERIAL AND METHODS OF STUDY

The patients were drawn from 331 consecutive admissions to Irvington House during the 26-month period beginning 1 July 1956. Of these patients, 44 had not had rheumatic fever and 22 had illnesses which had been originally labeled rheumatic fever but which did not fulfill the modified Jones criteria[2] for this diagnosis. The remaining 265 patients all had had unequivocal attacks of rheumatic fever and constitute the subjects of this report.

They were admitted to Irvington House at different phases of the acute rheumatic attack and in different stages of various kinds of anti-inflammatory therapy. Some arrived early in the attack while receiving no treatment or full doses of anti-inflammatory drugs, while others were sent purely for convalescence after all therapy had been stopped.

The routine management at Irvington House was as follows: Rectal temperature and "sleeping" pulse rate were measured daily. The uncorrected* (Wintrobe) erythrocyte sedimentation rate and serum C-reactive protein were tested at weekly intervals for at least eight weeks after both of these acute phase reactants had become and remained normal following cessation of anti-inflammatory therapy. All patients received continuous oral or parenteral prophylaxis** against streptococcal infections. These

* Wintrobe[30] no longer recommends correction of the sedimentation rate for changes in hematocrit.

** Grateful acknowledgement for the supply of these agents is given to Lederle Laboratories (for sulfadiazine), to E. R. Squibb and Sons (for oral penicillin "pentids"), and to Wyeth and Co. (for repository penicillin, "bicillin").

infections were sought by bacteriological and serological techniques. Throat cultures were taken for three consecutive days after admission and repeated thereafter when clinically indicated. The anti-streptolysin O titer was measured on admission and at monthly intervals until discharge. When there was clinical, bacteriological, or serological suspicion of streptococcal infections, consecutive sera were simultaneously tested for anti-streptolysin O, antistreptokinase, and anti-hyaluronidase titers.[17] Periodic dental examinations and appropriate local dental therapy helped maintain asepsis of the teeth and gums and removed dental inflammation as a potential cause[10] of fever and infection.

Since the patients came from many different sources, their initial therapeutic management was varied and could not be statistically randomized. After they reached Irvington House, the subsequent treatment depended on their clinical status and laboratory tests. When uncontrolled rheumatic activity was present, medication was begun or altered until the inflammation was suppressed. When the disease was under therapeutic control, the dosage of the anti-inflammatory agent was gradually lowered and discontinued after varying lengths of time, depending on the nature and duration of previous treatment and the condition of the patient.

The details of each patient's clinical and laboratory data at the referring institutions were added to the Irvington House records to obtain a chronology of the complete course of each rheumatic attack, from onset to the end of convalescence. These data were then searched for evidence of a rheumatic rebound. A rebound was defined as the reappearance of clinical or laboratory features of rheumatic activity after these manifestations had originally subsided, in the absence of an intercurrent Group A streptococcal infection. If abnormal vital signs and acute phase reactant values had not previously become normal, neither their reappearance nor the occurrence of clinical abnormalities was considered a rebound. This definition thus excluded patients in whom the original treatment may have been inadequately suppressive and it also permitted consideration of the rebound in situations where no suppressive treatment was used.

In a patient with recent unequivocal rheumatic fever and without an intercurrent streptococcal infection, the reappearance of rheumatic activity was defined according to the following criteria of abnormality:

*Laboratory criteria*

1. An uncorrected (Wintrobe) erythrocyte sedimentation rate of greater than 20 mm./hr.
2. Serum C-reactive protein of trace or more.

*Clinical criteria*

1. A temperature of greater than 100.4° F., rectally, occurring for three consecutive days (unless treatment intervened).
2. "Sleeping" pulse rate higher than 90 per minute, not associated with fever, occurring for three consecutive days (unless treatment intervened).
3. Recurrence of significant joint symptoms or signs.
4. The appearance of diastolic murmurs or of pericardial friction rubs, when these were unequivocal and were clearly new developments.
5. The development of congestive heart failure.
6. The appearance of nodules or erythema marginatum.

The pulse rate measurements were taken while the patients were asleep, to ensure that tachycardia was not due to psychic stimulation. The occurrence or reappearance of chorea was not listed as a specific manifestation of rheumatic activity, because the precise time of onset or cessation of choreic movements is difficult to determine, and because they often occur as a late manifestation of the same initial attack.[18, 19] Prolongation of the P-R interval was omitted from these parameters of rheumatic activity because of difficulties in its accurate graphic measurement,[21] uncertainties about the range of normality permitted at different cardiac rates,[1] the absence of correlations between its prolongation and other features of carditis,[8] and finally, because it sometimes remains permanently prolonged without relation to the rheumatic course.[5]

Using the above criteria for rheumatic activity, the following classification of rebounds could be made:

1. No rebound.
2. Slight "laboratory rebound": CRP trace and/or ESR 21-30 mm./hr.
3. Significant "laboratory rebound": CRP 1+ or higher and/or ESR of over 30 mm./hr.
4. Slight "clinical rebound": laboratory indications or rebound, together with fever.
5. Significant "clinical rebound": laboratory indications of rebound, with or without fever, together with any of the other clinical criteria cited above.

Clinical features had to be associated with laboratory abnormalities in order to be classified as a "clinical rebound." This specification avoided difficulties in evaluating the cause of minor arthralgias and other subjective complaints, and in analyzing nonspecific changes in the intensity of established auscultatory phenomena. Untreated fever or nocturnal tachycardia had to be present for three days before the assumption that they were part of a clinical rebound. This specification eliminated the sporadic, isolated rises in temperature and pulse which occur so frequently in hospitalized patients. Clinical or laboratory abnormalities were attributed to a rebound only when there were no alternative likely causes—such as nonspecific respiratory illnesses, transient reactions to injections of benzathine penicillin,[9] etc.

For the analysis of anti-inflammatory therapy the patients were divided into four groups:

1. *No treatment.* These patients had received no anti-inflammatory therapy or had been given only sporadic, isolated doses.
2. *Salicylate treatment.* These patients had received a definite course of some salicylate preparation, generally aspirin.
3. *Steroid treatment.* These patients had received steroids exclusively, the most commonly used agents being cortisone, prednisone, and/or ACTH.
4. *Combined treatment.* These patients had received salicylates and steroids together, the combinations having been given in many different forms. All patients in this group had received at least ten days of salicylate and at least five days of steroid therapy. (Patients on steroids who had previously received aspirin for less than ten days were classified with those who received steroids alone.) In 23 patients the two drugs were started together, the administration of salicylate continuing after steroid was stopped. In 13 patients steroid was added after salicylate was

started, and was continued beyond the cessation of salicylate therapy. The remaining 13 patients in the "combined" group received the two drugs sequentially or concomitantly in a variety of patterns. A rebound was associated with "combined" therapy only if the patient had previously received both types of treatment.

For classification of the intial clinical status, the patients were divided as follows: Those with "no valvular involvement" had had only arthritis or chorea but lacked the diastolic murmurs or significant apical systolic murmurs which characterized those with "valvular involvement."[2,3] The distinction between physiological and pathological apical systolic murmurs was made according to previously described criteria of loudness, length, and location.[4,5] In the group with valvular involvement, patients with significant heart enlargement (i.e., at least one chamber 2+ enlarged) were separated from those with no or slight cardiomegaly.

TABLE 1. TYPES OF REBOUNDS IN RELATION TO PRECEDING THERAPY
(THE FIGURES SHOW THE NUMBER OF PATIENTS IN EACH CATEGORY)

| | *Type of rebound* | | | | | |
|---|---|---|---|---|---|---|
| | *Laboratory* | | | *Clinical* | | |
| *Type of therapy* | *None (1)* | *Slight (2)* | *Significant (3)* | *Slight (4)* | *Significant (5)* | *Total* |
| Salicylate | 52 | 15 | 29 | 1 | 9 | 106 |
| Steroid | 25 | 14 | 12 | 3 | 23 | 77 |
| Combined | 21 | 8 | 6 | 4 | 9 | 48 |
| None | 22 | 6 | 6 | 0 | 0 | 34 |
| Total | 120 | 43 | 53 | 8 | 41 | 265 |

## RESULTS

### *Types of rebound and relation to previous treatment*

The following features may be noted from Table 1:

1. A rebound occurred in more than half the patients (145/265). In the individual treatment groups, a rebound occurred in the following percentages: salicylates, 51 per cent (54/106); steroid, 68 per cent (52/77); combined, 56 per cent (27/48); and no treatment, 35 per cent (12/34).

2. There were 43 slight and 53 significant laboratory rebounds. They occurred even in patients who had received no anti-inflammatory therapy, appearing in more than one third (12/34) of the "untreated" group.

3. Of the 12 "untreated" patients with laboratory rebounds, 5 had no heart disease and 7 had valvular involvement without significant cardiomegaly. These rebounds occurred: during early ambulation after strict bedrest (in 4); in the first two weeks after transfer to the ambulatory unit

(in 5); beyond two weeks after transfer to the ambulatory unit (in 3). Since the progression to ambulation was often made as soon as the acute phase reactants reached normal values, it is to be expected that some elevations in these values would occur spontaneously while the patients were ambulatory. These data therefore do not permit the conclusion that the laboratory rebounds in this group necessarily were due to the ambulation.

4. There were 8 slight and 41 significant clinical rebounds. In patients who received suppressive treatment, clinical rebounds occurred with the following frequency: salicylates, 9 per cent (10/106); steroid, 34 per cent (26/77); and combined treatment, 27 per cent (13/48). No clinical rebounds occurred in the untreated patients.

TABLE 2. TYPE OF TREATMENT IN RELATION TO INITIAL CLINICAL STATUS (THE FIGURES SHOW THE NUMBER OF PATIENTS IN EACH CATEGORY)

| *Clinical status* | *Type of treatment* | | | | |
|---|---|---|---|---|---|
| | *None* | *Salicylate* | *Steroids* | *Combined* | *Total* |
| No valvular involvement | 19 | 48 | 28 | 19 | 114 |
| Valvular involvement without significant heart enlargement | 14 | 49 | 33 | 19 | 115 |
| Valvular involvement with significant heart enlargement | 1 | 9 | 16 | 10 | 36 |
| Total | 34 | 106 | 77 | 48 | 265 |

These data indicate that a laboratory or clinical rebound (as herein defined) is a common event in acute rheumatic fever, occurring in more than half of the patients in this survey. Laboratory rebounds occurred even in patients who received no anti-inflammatory therapy; clinical rebounds occurred only in patients who received anti-inflammatory therapy and were significantly more frequent in those whose treatment included steroids than in those who received salicylate alone, ($X^2 = 13.4$; $p < 0.01$).

*Types of treatment and relation to clinical status of patients* (Table 2)

In patients with little or no cardiac damage, salicylates alone were used as frequently as steroids and combined therapy. In patients with significant cardiomegaly, the number who received steroid and combined treatment was almost three times as great as those who were given aspirin alone. The greater use of steroids in the latter group of patients may be due to two fea-

tures. First, many physicians continue to prefer steroids for acutely ill patients with significant heart disease even when no cardiac failure is present. Secondly, when it was believed that carditis was not adequately controlled by salicylates alone, steroids were added to the treatment. In this situation the patient's therapy would be classified in the steroid or combined group.

*Types of rebound and relation to clinical status of patients* (Table 3*)

Of the patients with no valvular evidence of heart disease, 54 per cent (62/114) had no rebound, while 12 per cent had a clinical rebound. Of

TABLE 3. TYPES OF REBOUNDS IN RELATION TO INITIAL CLINICAL STATUS OF PATIENTS
(THE FIGURES SHOW THE NUMBER OF PATIENTS IN EACH CATEGORY)

| *Clinical status* | *No rebound* | *Laboratory rebound* | *Clinical rebound* | *Total* |
|---|---|---|---|---|
| No valvular involvement | 62 | 38 | 14 | 114 |
| Valvular involvement without significant heart enlargement | 53 | 43 | 19 | 115 |
| Valvular involvement with significant heart enlargement | 5 | 15 | 16 | 36 |
| Total | 120 | 96 | 49 | 265 |

those who had valvular heart disease without significant cardiac enlargement, 46 per cent (53/115) had no rebound, 17 per cent had a clinical rebound. Of the patients with significant cardiac enlargement, only 14 per cent (5/36) were free of rebound, while clinical rebounds occurred in 44 per cent (16/36). These data are statistically significant ($X^2 = 17.3$, $p < 0.01$) and suggest that an increase in the severity of heart disease predisposes to an increased incidence of clinical rebound. However, as Table 2 indicates, steroid treatment was used more often in patients with severe heart disease, and the higher incidence of clinical rebounds in this group might possibly be related to the therapy rather than to the cardiac status. This possibility is explored in Table 4, below.

* For this and subsequent tables, the designation "laboratory" and "clinical" rebounds combines the "slight" and "significant" sub-groups of each category.

*Types of rebounds and relation to clinical status and preceding treatment* (Table 4)

In patients with no valvular involvement, a clinical rebound occurred in 6 per cent of those treated with salicylate, in 18 per cent of the steroid group, and in 32 per cent of those who received combined therapy. For these patients, the lower incidence of clinical rebounds after salicylates as compared to steroids and combined therapy is statistically significant ($X^2 = 4.75$, $p < 0.05$). In patients with evidence of valvular disease but without significant cardiomegaly, a clinical rebound occurred in the treatment groups

TABLE 4. INCIDENCE OF CLINICAL REBOUNDS IN RELATION TO PRECEDING TREATMENT AND INITIAL CLINICAL STATUS

| *Initial clinical status of patients* | *Therapeutic agent* | *Number of patients* | *Number with clinical rebounds* | *Percentage* |
|---|---|---|---|---|
| No valvular involvement | None | 19 | 0 | 0% |
| | Salicylate | 50 | 3 | 6% |
| | Steroids | 28 | 5 | 18% |
| | Combined | 19 | 6 | 32% |
| Valvular involvement without significant heart enlargement | None | 14 | 0 | 0% |
| | Salicylate | 48 | 5 | 10% |
| | Steroids | 33 | 12 | 36% |
| | Combined | 19 | 2 | 11% |
| Valvular involvement with significant heart enlargement | None | 1 | 0 | 0% |
| | Salicylate | 8 | 2 | 25% |
| | Steroids | 16 | 9 | 56% |
| | Combined | 10 | 5 | 50% |

as follows: salicylates, 10 per cent; steroids, 36 per cent; combined therapy, 11 per cent. For these patients the incidence of clinical rebounds after salicylates is significantly lower than that after steroids alone ($X^2 = 7.94$; $p < 0.01$) and somewhat less significantly lower ($X^2 = 3.41$; $p < 0.10$) when compared against the total of steroid and combined therapy. In patients who had significant cardiac enlargement, clinical rebounds occurred in the treatment groups as follows: salicylates, 25 per cent; steroids, 56 per cent; combined, 50 per cent. For these patients, the post-salicylate clinical rebound percentages are *not* significantly lower than the others ($X^2 = 1.05$; $p < 0.3$).

The partial superiority of salicylates suggested by these results must be viewed with reservation. In the initial selection of treatment, patients who were more severely ill were often given steroids immediately. Patients

treated with salicylates who failed to show a suitable clinical response usually had steroids added to the therapy. Patients with "chronic" or unusually prolonged rheumatic activity, whose acute phase reactants never reached normal values under salicylate therapy, either received additional steroid treatment or did not qualify for classification in the "rebound" group according to the criteria of the present study. Thus the patients of this survey who

TABLE 5. INCIDENCE OF CLINICAL REBOUNDS IN RELATION TO INITIAL CARDIAC STATUS AND LENGTH OF ANTI-INFLAMMATORY TREATMENT
(In each instance, the denominator gives the total number of patients in each group, and the numerator gives the number who had clinical rebounds)

| *Agent* | *Initial cardiac status* | *Weeks of treatment* | | | | |
|---|---|---|---|---|---|---|
| | | *0-4* | *4-8* | *8-12* | *over 12* | *Total* |
| Salicylate | No valvular involvement | 1/12 | 2/26 | 0/10 | 0/2 | 3/50 |
| | Valvular involvement without significant heart enlargement | 3/11 | 2/24 | 0/10 | 0/3 | 5/48 |
| | Valvular involvement with significant heart enlargement | 1/2 | 1/4 | 0/1 | 0/1 | 2/8 |
| | Total | 5/25 | 5/24 | 0/21 | 0/6 | 10/106 |
| Steroid | No valvular involvement | 1/8 | 2/16 | 0/1 | 2/3 | 5/28 |
| | Valvular involvement without significant heart enlargement | 0/2 | 3/16 | 3/8 | 6/7 | 12/33 |
| | Valvular involvement with significant heart enlargement | 0/0 | 2/5 | 3/7 | 4/4 | 9/16 |
| | Total | 1/10 | 7/37 | 6/16 | 12/14 | 26/77 |

received only salicylates were more likely to be those who had a relatively benign clinical course. In those whose therapeutic response to salicylate was not ideal, steroids were often added to the treatment and any subsequent rebounds were then attributed to "combined" therapy, rather than to salicylates alone.

*Types of rebound and relation to duration of preceding treatment* (Table 5)

The after-effects of therapy may be influenced by its dosage and duration. In considering the relation of these variables to subsequent rebounds, only the salicylate and steroid groups could be analyzed because "combined"

therapy had been given in too many different ways to permit detailed classification. The occurrence of rebounds was tabulated in both the steroid and salicylate groups with regard to *total* dosage of the agent (sum of the daily doses) and the total duration of treatment. In addition, treatment in salicylate patients was classified, using body weight, into units of total dosage per pound. No striking correlations emerged with either agent, from a consideration either of total dosage or of total dosage per pound. The data on *duration* of treatment seemed to be more significant and are listed in Table 5. In patients who received salicyates, clinical rebounds occurred,

TABLE 6. INCIDENCE OF CLINICAL REBOUNDS IN RELATION TO AGE AND SEX OF PATIENTS AND TREATMENT USED
(In each instance, the denominator gives the total number of patients in each group, and the numerator gives the number of those who had clinical rebounds)

| | | *Treatment used* | | | | |
|---|---|---|---|---|---|---|
| | *Age (years)* | *None* | *Salicylate* | *Steroid* | *Combined* | *Total* |
| Boys | 4-9 | 0/1 | 3/20 | 6/15 | 3/7 | 12/43 (28%) |
| | 10-14 | 0/12 | 3/41 | 10/26 | 7/19 | 20/98 (20%) |
| | >14 | 0/2 | 1/11 | 0/3 | 2/4 | 3/20 (15%) |
| | Total | 0/15 | 7/72 | 16/44 | 12/30 | 35/161(22%) |
| Girls | 4-9 | 0/9 | 0/13 | 7/16 | 0/9 | 7/47 (15%) |
| | 10-14 | 0/8 | 3/16 | 3/14 | 1/9 | 7/47 (15%) |
| | >14 | 0/2 | 0/5 | 0/3 | ... | 0/10 (0%) |
| | Total | 0/19 | 3/34 | 10/33 | 1/18 | 14/104(13%) |

irrespective of cardiac status, only in those treated less than 8 weeks. In patients who received steroids, the incidence of clinical rebounds increased as the duration of treatment increased: they appeared in 10 per cent (1/10) of those treated for 0-4 weeks, in 19 per cent (7/37) of the 4-8 week group, and 38 per cent (6/16) of the 8-12 week group, and in 86 per cent (12/14) of those treated for more than 12 weeks. This relationship is statistically significant ($X^2 = 15.7$, $p < 0.01$). This table also shows that post-steroid clinical rebounds (as noted in Table 4) were increasingly likely as the severity of heart disease was increased.

These results indicate that steroid treatment, given for more than 8 weeks, was associated with clinical rebounds in more than half of the patients, particularly in those with more severe cardiac damage. Salicylate treatment maintained for longer than 8 weeks appeared to prevent clinical rebounds,

regardless of cardiac status, but the present survey included only 2 patients with significant cardiomegaly who received salicylate alone for over 8 weeks. Clinical rebounds in patients in this category have been noted previously at Irvington House and elsewhere, and the present data do not imply that rebounds will always be prevented by long-term salicylate treatment.

*Relation of clinical rebounds to age and sex* (Table 6)

Clinical rebounds occurred in 35 (22 per cent) of the 161 boys in this series, and in 14 (13 per cent) of the 104 girls. Despite the tendency of steroids to produce striae, acne, and other cutaneous changes, hormonal

TABLE 7. INTERVAL BETWEEN CESSATION OF TREATMENT AND OCCURRENCE OF REBOUND
(THE FIGURES SHOW THE NUMBER OF PATIENTS IN EACH CATEGORY)

| *Time of occurrence of rebound after cessation of treatment* | *Type of rebound* | |
|---|---|---|
| | *Laboratory* | *Clinical* |
| During tapering to 1st week after | 26 | 39 |
| 1-2 weeks | 14 | 4 |
| 2-4 weeks | 23 | 3 |
| 4-8 weeks | 20 | 3 |
| Beyond 8 weeks | 13 | 0 |

therapy was used in 49 per cent of the girls (51/105) compared to 46 per cent (74/161) of the boys. The present data do not confirm a previous observation[10] that clinical rebounds were most prevalent in teen-age girls. At all three age groupings in the present survey, clinical rebounds occurred more often in boys than in girls, and the likelihood of their appearance was less as the boys grew older. In the girls the incidence was the same in the age group 4-9 as in the age group 10-14, and no clinical rebounds occurred in girls over 14 years of age. None of these age-sex differences has statistical significance, however.

*Relation of rebounds to penicillin treatment*

Recently, it has been suggested[14] that large doses of penicillin, given *after* the onset of the rheumatic attack, may reduce the residual cardiac damage. This hypothesis has not been tested in the present study. However, the relationship of penicillin treatment to subsequent clinical rebounds could be considered. Of 130 patients who received only sporadic penicillin treatment or none, clinical rebounds occurred in 26 (20 per cent). Of those who definitely received a streptococcal-eradicating course of penicillin, clinical

rebounds occurred in 11 per cent (8/74). This difference in the two groups is not significant, however, since $X^2 = 2.25$, with $p > 0.1$. It should be noted that Mortimer *et al.*[14] found no difference in frequency of rebounds in the treated and untreated groups in their study of massive penicillin therapy.

*Time interval between end of treatment and occurrence of rebounds* (Table 7)

In patients with laboratory rebounds, the abnormal acute phase reactants appeared sporadically following their original return to normal levels. Although more laboratory rebounds occurred during the first week than at any other time after cessation of therapy, they also occurred in appreciable frequency at subsequent periods. Of the laboratory rebounds, 86 per cent had occurred by the end of eight weeks while 14 per cent took place beyond eight weeks. In contrast, most (80 per cent; 39/49) of the clinical rebounds occurred while therapy was being tapered or during the first week after it was stopped. No clinical rebound occurred eight weeks or more after therapy was stopped. Thus, patients who did not have a clinical rebound within a week or two after cessation of therapy were likely to complete their course without having one, and would not have one after eight weeks. On the other hand, elevations of acute phase reactants (a laboratory rebound) could occur anytime within the first two post-therapeutic months and appeared even later in 14 per cent of patients.

*Features and sequelae of the rebounds*

Figures 1 and 2 illustrate the sequence of events in two patients with clinical rebounds. It is of interest that laboratory rebounds subsided spontaneously in all cases and had no subsequent clinical effects. The details of the events, management, and sequelae of clinical rebounds are described separately.[38] In the 49 clinical rebounds of the present group, new cardiac damage did not occur in 14 patients who initially had no valvular involvement. Of the 35 patients who originally had carditis, 10 developed new cardiac events and in 2, the damage seemed permanent. The incidence of new cardiac damage in the 35 clinical rebounds of this group was related to previous treatment as follows: salicylate, 4 in 7; steroids, 5 in 21; combined, 1 in 7. These numbers are too small for comparative statistical significance.

It should be noted that the incidence of clinical rebounds and of individual events within them is somewhat less in the present series than in those reported previously. Some of these differences are probably due to the criteria used to define the rebound, and the demand in the present study that values for acute phase reactants had to have achieved normal limits before the reappearance of abnormal features could be considered a true rebound.

## DISCUSSION

A disadvantage of the present analysis is that, like most other studies of the rebound phenomenon, it is retrospective; the therapeutic agents were not chosen randomly and were not always maintained according to a pre-

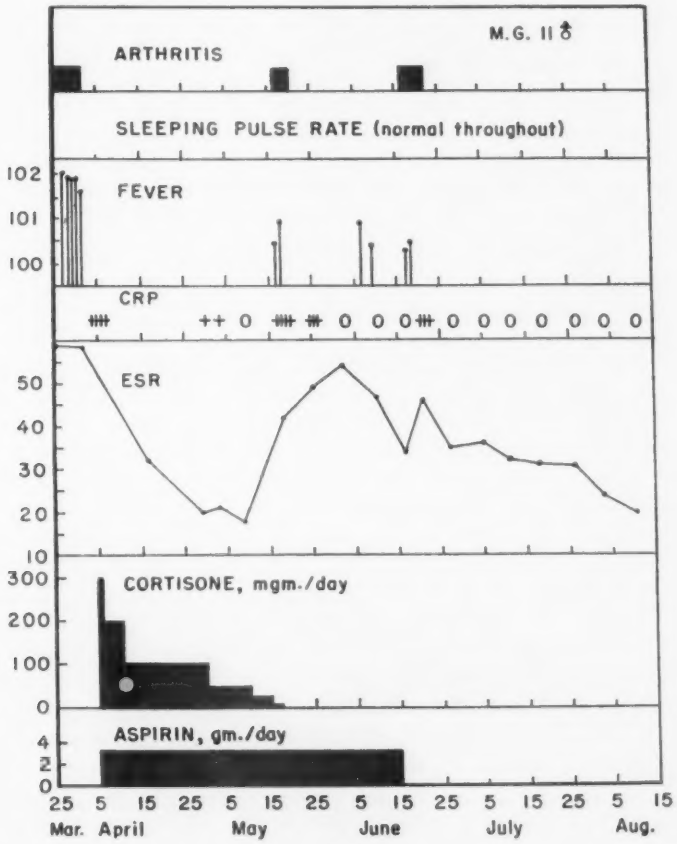


FIG. 1. *Two clinical rebounds with first attack of rheumatic fever in an 11-year-old boy.* The initial arthritis and fever had subsided before admission to hospital. Combined therapy was begun because of a systolic murmur, loudest along the left sternal border, and positive acute phase reactants. After all abnormalities subsided, fever and arthritis returned when cortisone was stopped and reappeared later when aspirin was stopped. The systolic murmur was finally thought to be physiological, and no other suggestions of carditis were found. Patient has remained free of heart disease.

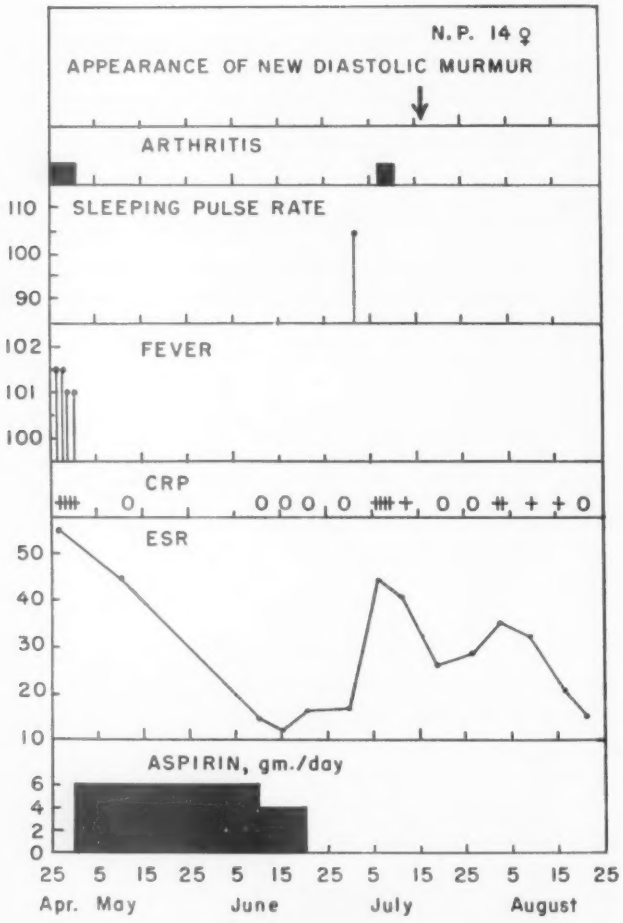


FIG. 2. *New cardiac features with a clinical rebound in a 14-year-old girl.* This first attack of rheumatic fever began with polyarthritis and a significant apical systolic murmur. All abnormalities subsided on salicylate therapy, which was stopped after four weeks. Within the next two weeks the patient developed fever, arthritis, and a basal blowing diastolic murmur, together with an elevated ESR and CRP. All features of the rebound subsided spontaneously in the next few weeks except for the diastolic murmur. Hemodynamic changes of aortic regurgitation did not occur, however, and the murmur disappeared in the next two years.

arranged plan. This same circumstance in the current survey, however, is advantageous because it provides the opportunity to compare the results of various therapeutic agents and the influence of different durations of treatment. These comparisons could not be made in studies where salicylates and/or steroids were given to all patients for the same length of time and where "combined" treatment had not been used. The present work has several other advantages: the patients were in the younger age group where rheumatic fever predominates, their number is several times greater than in any previous reports, an untreated group was available for study, and the rebounds were related to the initial clinical status of the patients. The division of patients into three clinical categories has helped to minimize the effects of nonrandom allocation of therapeutic agents and has enabled the recognition of direct correlations between the cardiac status and the occurrence and types of clinical rebounds.

The results show that rebounds are a common event in acute rheumatic fever; they appear in two ways, each with different consequences. The laboratory rebound consists only of abnormalities in acute phase reactants of the blood. It subsides in all patients without additional therapy and it apparently does not affect the patients' clinical status. By contrast, the clinical rebound is more to be feared because of its acute, and potentially permanent, cardiac effects. Although clinical rebounds did not bring carditis to patients who did not have it initially, they did produce significant new cardiac manifestations in some of those who had previously shown evidence of valvular involvement. The laboratory rebounds were unrelated to clinical status and preceding therapy; they could be found even in untreated patients. The clinical rebounds were more frequent with increasing severity of heart disease, and occurred only in patients who had received anti-inflammatory therapy; they were particularly common after treatment with steroids.

The implications of these data can be applied to a number of basic and clinical problems in rheumatic fever. Some of these will be considered separately.[28] The remainder of the discussion here will be devoted to possible mechanisms of the rebound phenomenon.

The clinical rebound patterns observed in this survey cannot be well explained by any of the three possible causes to which this phenomenon is usually attributed. *Polycyclic rheumatic fever*[29] is unlikely because the incidence of rebounds is far higher than the natural occurrence of multiple rheumatic cycles and because the rebounds occur too close to the time of reduction or cessation of therapy. *The persistence of rheumatic activity* due to premature withdrawal of treatment[6] does not seem a valid explanation because so many rebounds occur after extremely long therapeutic courses. *Relative*

*adrenal insufficiency*[27] does not account for the rebounds which follow salicylate treatment, and hypoadrenalism has seldom been demonstrable in rebounds after steroid treatment.[28] Moreover, none of these mechanisms would account for the increased incidence of clinical rebounds as the severity of cardiac damage increases.

An attractive explanation which fits the observed data is provided by a new hypothesis, which may conveniently be called "suppressed inflammation." This theory assumes that the average attack of rheumatic fever creates a fixed amount of inflammation which must be dissipated. The amount of inflammation will differ in different patients. Its total quantity will determine the severity of the clinical features. The rapidity with which it appears will determine the peak of its intensity and the rapidity with which it dissipates will determine the duration of the attack. Anti-inflammatory therapy will suppress the appearance and dissipation of the inflammation. The suppression can be total or partial, depending on the amount of inflammation and the therapy employed.

If the suppression is total, as with full doses of steroids, the inflammation remains dormant and accumulates until the therapy is reduced or stopped. At that time, the amount of residual inflammation and the rapidity of its appearance will determine whether the resultant flare-up of rheumatic activity is more or less severe than the original attack. Longer periods of *totally suppressive* therapy will result in greater quantities of residual inflammation and hence greater likelihood of post-therapeutic clinical rebounds.

If the suppression is partial, as with salicylates, some inflammation can continue to dissipate during the period of treatment although the amount of inflammation which appears may not be great enough to create clinical symptoms. In this situation, longer durations of therapy would be desirable since less residual inflammation is likely to remain when the therapy is stopped.

Figure 3 (A-F) shows hypothetical clinical situations to illustrate the data and the theory derived from them. The course of rheumatic fever in a typical untreated patient is shown in A. The inflammation first rises to the level of clinical abnormalities, then drops to the level of only laboratory abnormalities and finally reaches normal levels. Drawing B shows how this course would be influenced by a long period of steroid treatment. The dotted line shows the natural course of the untreated disease, and the solid line shows the effect of the therapy. The steroids suppress inflammation promptly and profoundly. The undispersed inflammation is shown in the dotted area. When the steroids are stopped, this inflammation erupts and a clinical rebound occurs. A long course of steroid therapy will not always be followed by a clinical rebound, because the original amount of inflammation

may have been small, or because some dissipation of inflammation may occur during the long "tapering" period at gradually lowered dosage. During the latter interval, incomplete suppression of inflammation may allow enough sub-clinical dispersion so that the cessation of treatment will be followed by a mild rebound or none. Figure C shows how the course of rheumatic activity is influenced by steroid treatment of shorter duration. During the period of total suppression, less inflammation accumulates so that the

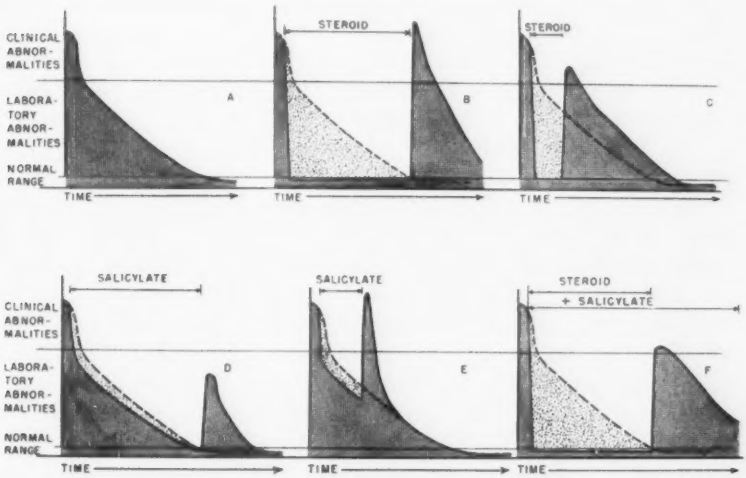


FIG. 3. *Schematic representation of the course of rheumatic activity as affected by different therapeutic techniques.* In each case, the solid black line shows the actual course under treatment and the dotted line shows the comparative course in the untreated patient. The heavily shaded area denotes active, overt rheumatic inflammation and the lightly shaded area shows suppressed rheumatic inflammation which later may become active. Drawing A shows the course in an untreated patient and the treatments in B-F are further described in the text.

subsequent rebound is milder. Figure D shows the effect of the less potent salicylates. These can only suppress inflammation partially to the level of laboratory abnormalities. This may not achieve ideal results in tests of acute phase reactants, but it allows the expression of considerable amounts of inflammation at a subclinical level. If the salicylate is continued for a long time, enough inflammation may dissipate during the treatment so that the post-therapeutic remainder is small enough to create little or no rebound. With a shorter course of salicylate, as in E, the residual inflammation

resumes its appearance at a higher level of disease activity, so that the rebounds may reach clinical levels of severity.

These observations will explain why an appropriate form of combined therapy may reduce the severity of clinical rebounds. With steroid treatment the suppressed inflammation accumulates as shown in the dotted area of F. During this period, the suppressant effect of salicylates appears superfluous. When the steroids are lowered and stopped, however, the dispersion of inflammation resumes. The presence of salicylates at this time will not prevent the flare-up from occurring but will help regulate its severity as may be seen in comparison with Figure B.

The above conjectures obviously do not account for all aspects of rheumatic activity, but appear to offer a satisfactory explanation for most of the observed clinical rebound phenomena.

## SUMMARY

The rebound phenomenon has been studied in 265 children and adolescents consecutively admitted to Irvington House during the acute or convalescent phases of unequivocal attacks of rheumatic fever. In each patient the analysis included a sustained chronological account of the illness from its onset to the end of convalescence. The patients were classified according to their initial cardiac status and the anti-inflammatory treatment which had been given. The rebound was defined as the reappearance, after initial subsidence, of clinical and/or laboratory manifestations of rheumatic activity, in the absence of an intervening streptococcal infection.

Laboratory rebounds were defined as the recurrence of abnormal values only in the erythrocyte sedimentation rate or serum C-reactive protein. They appeared in 96 patients and all subsided spontaneously without any evident adverse clinical effects. Their incidence did not seem related to the clinical status or to the nature of the preceding treatment and they also occurred spontaneously in patients who received no suppressive treatment. In treated patients, they appeared at varying intervals after therapy was stopped and 14 per cent occurred later than 8 weeks.

A clinical rebound, manifested by fever and/or joint pains and/or new cardiac signs, together with laboratory abnormalities, occurred in 49 patients. In 39, it appeared while treatment was being reduced or during the first post-therapy week. These rebounds did not bring clinical evidence of heart disease to patients who were initially free of it. New cardiac damage, however, developed in 10 and persisted in 2 of the 35 clinical rebounds in patients who initially had valvular involvement. A clinical rebound occurred more frequently in patients who had valvular damage than in those

who did not, and was particularly common in those with significant cardiac enlargement. There were no clinical rebounds in patients who received no anti-inflammatory therapy. In patients who received suppressive therapy, clinical rebounds were more prevalent in those given steroids or combined steroid-salicylate treatment than in those treated with salicylate alone. In the salicylate-treated group, the incidence of clinical rebounds was unrelated to cardiac status and they occurred exclusively in those patients who received therapy for less than 8 weeks. In the group treated with steroids the incidence of clinical rebounds increased markedly as the duration of treatment increased and was particularly high in patients with substantial initial cardiac damage. "Combined" therapy was associated with approximately the same incidence of clinical rebounds as steroid treatment alone, but the rebounds seemed less severe.

Of the various hypotheses which have been advanced for the pathogenesis of most clinical rebound phenomena, the most satisfactory explanation appears to be that suppressive medication prevents the dispersion of rheumatic inflammation and that the accumulated residual inflammation then appears in the form of a rebound when the suppression is reduced or stopped. The severity of the rebound will depend upon how much inflammation was present initially, how much of it was suppressed by the therapy, and how much remains afterwards.

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MARIO SPAGNUOLO*
ALVAN R. FEINSTEIN**

*Irvington House, Irvington-on-Hudson, New York, and the Department of Medicine, New York University School of Medicine, New York*

# THE REBOUND PHENOMENON IN ACUTE RHEUMATIC FEVER
## II. TREATMENT AND PREVENTION†

The post-therapeutic rebound phenomenon in acute rheumatic fever may include clinical features or may consist only of abnormalities in laboratory tests. A survey[6] of these events has shown that "laboratory" rebounds were invariably benign, did not correlate with preceding therapy or with cardiac status, and subsided spontaneously. In contrast, the "clinical" rebounds were sometimes worse than the manifestations of the original attack, had an increased incidence with increasing severity of heart disease, occurred more frequently in patients who received steroids than in those who did not, and carried the threat of additional cardiac damage.

The prevention of clinical rebounds has been attempted by several therapeutic designs. Most designs relate to the length, dosage, and supplements of steroid therapy, and the use of salicylates alone is seldom considered. In one plan[10, 13, 15, 16] steroid treatment is continued for a long time with an extended period of gradual reduction before it is stopped. In another[26] the steroids are given at high dosage but only for one or two weeks. A third approach has added salicylates to the steroids and continued salicylate treatment after the steroids are discontinued.[9, 12, 18] Within their basic structure, these three therapeutic plans have numerous minor variations. No studies have compared the three programs, and their relative effectiveness is uncertain.

Other uncertainties and controversies surround the management of rebound phenomena. At one extreme is the belief[2, 21] that suppressive therapy should be resumed whenever a rebound occurs, regardless of its clinical or laboratory manifestations. At the other extreme is the implication[6] that additional treatment is often undesirable because further suppression of the rheumatic inflammation may result in recurrent rebounds when the treat-

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* Resident Physician, Irvington House.

** Medical Director, Irvington House; Assistant Professor of Medicine, New York University School of Medicine.

† Supported in part by Fellowship from the New York State Department of Health.

*Received for publication 4 June 1960.*

ment is stopped. Between these extremes are other treatment programs based upon specific aspects of the clinical situation.

In an attempt to clarify some of these problems, the present study has analyzed the management and sequelae of rebounds and methods for preventing rebounds.

## CLINICAL MATERIAL AND METHODS

The criteria for rheumatic activity, laboratory rebounds and clinical rebounds, and the classification of the patients' clinical status were described in detail previously.[4] The basic population of the present report consists of the same 265 children and adolescents with acute rheumatic fever who were studied previously. They represented all the patients whose attacks fulfilled the modified Jones diagnostic criteria[1] and who were admitted to Irvington House consecutively from 1 July 1956 to 31 August 1958. In 96 patients of this group laboratory rebounds occurred and their sequelae have been described here. In 49 patients clinical rebounds occurred. To obtain more clinical rebounds for this study, an additional 15 patients were added to this original group. The extra 15 patients represented all the clinical rebounds encountered in the 150 consecutive patients with unequivocal rheumatic fever admitted to Irvington House from 1 September 1958 to 30 August 1959. Thus, the 64 patients whose clinical rebounds have been analyzed below in Figures 4 to 6 and Tables 1 to 3 comprise all the instances of this event in 415 consecutive cases of rheumatic fever seen in a 3-year period ending 30 August 1959.

The prevention of post-steroid clinical rebounds was attempted, whenever possible, in all patients admitted to Irvington House after November 1958. The previous study of rebounds[8] had been completed at that time, and its conclusions suggested that the incidence of post-steroid clinical rebounds might be reduced by the concurrent use of salicylates. Accordingly, in patients who arrived at Irvington House while receiving steroids alone, salicylates were usually added to the program when the reduction and tapering of steroids began, and salicylates were continued after the steroids were stopped. The success of this form of "overlap" treatment in preventing clinical rebounds has been tabulated here (Table 4) and compared against results obtained with another form of combined therapy in which steroids and salicylates are begun simultaneously. Although these different groups of patients were studied consecutively rather than concomitantly, they were quite similar in regard to the other major parameters of the rheumatic attacks and differed significantly only in the type of anti-inflammatory therapy given in the *late* stages of their treatment.

## RESULTS

### *Sequelae and management of laboratory rebounds*

None of the original laboratory rebounds was treated; an additional (secondary) laboratory rebound occurred spontaneously after subsidence of the initial abnormalities in 19 of the 96 patients. All the secondary laboratory rebounds also subsided spontaneously, and had no apparent effect on the clinical status. The effects of anti-inflammatory treatment in a situation

resembling a laboratory rebound are shown in Figure 1. This patient's initial clinical features were arthritis without valvular involvement. He was treated with salicylates and experienced a *clinical* rebound when the therapy was stopped. The clinical features then subsided spontaneously, but the acute phase reactants were still abnormally elevated. At this time, while the

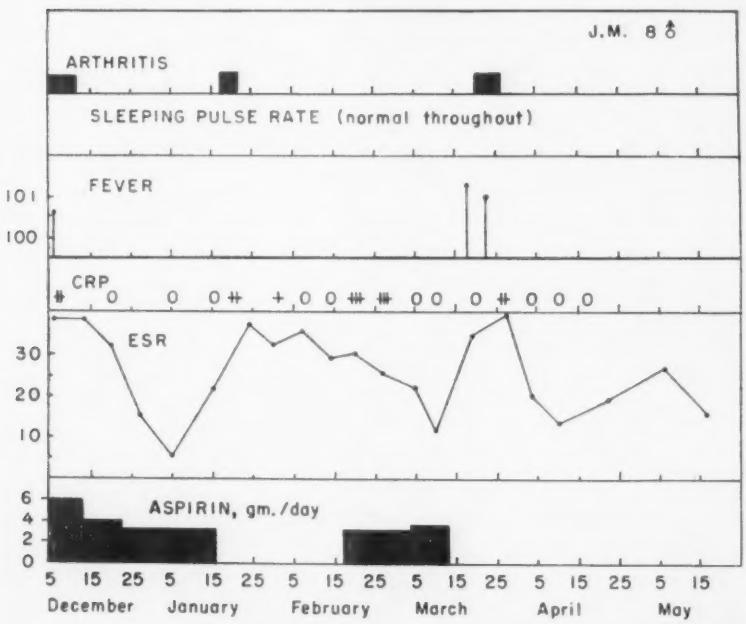


FIG. 1. *Occurrence of a clinical rebound after salicylate therapy given at time of abnormalities only in laboratory tests.* The first attack of rheumatic fever in this 8-year-old boy produced polyarthritis without carditis and responded well to salicylate treatment. When the latter was discontinued after three weeks, arthritis recurred. It had subsided spontaneously but acute phase reactants were still abnormal when the patient received two weeks of aspirin for investigative purposes. Cessation of the aspirin was followed by a clinical rebound with arthritis, fever, and laboratory abnormalities. (See text.)

patient had only laboratory abnormalities, a course of aspirin therapy was given as part of a study of the effect of salicylate upon serum transaminase.[34] When the latter course of aspirin was stopped, the patient then had another clinical rebound. This unexpected result confirms a previous observation[35] that the use of suppressive treatment for laboratory abnormalities may be

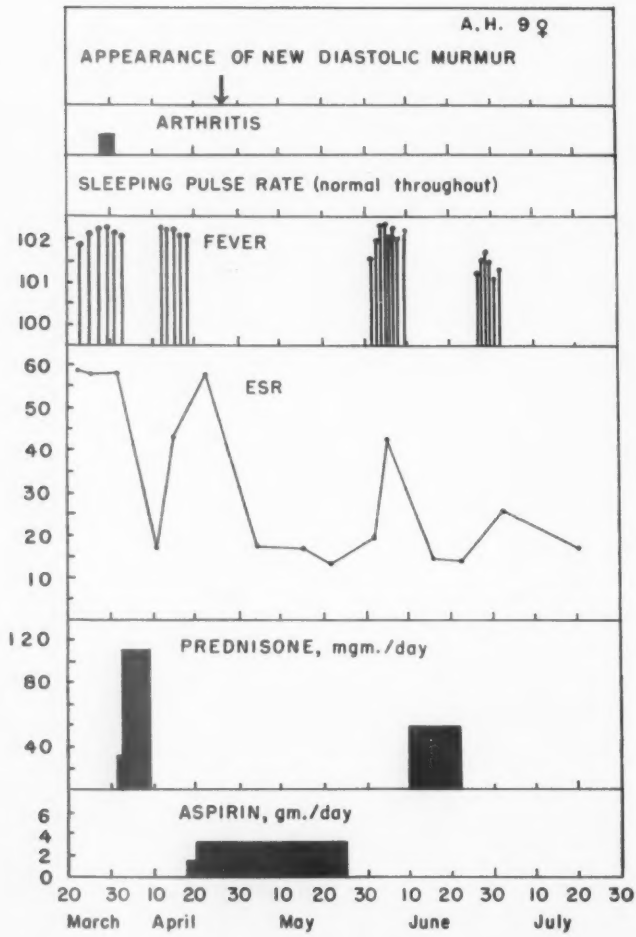


FIG. 2. *Appearance of new cardiac features and recurrence of clinical rebounds after each course of suppressive treatment.* This 9-year-old girl, in her first attack of rheumatic fever, had polyarthritis, fever, positive acute phase reactants, and significant apical systolic and mid-diastolic murmurs, without cardiomegaly. With high dosage of prednisone, all these abnormalities disappeared promptly. The cessation of prednisone, one week later, was followed by fever and later by a new basal diastolic murmur. The latter persisted despite aspirin therapy. The fever whch reappeared when aspirin was stopped subsided when prednisone was resumed and returned when prednisone was stopped. It then subsided spontaneously but the basal diastolic murmur has persisted.

followed by adverse effects, and the observation has served as background for subsequent experimental studies of rebound phenomena.[7]

*Sequelae of clinical rebounds*

Figures 2 and 3 give illustrative examples of the detailed course of events in the clinical rebounds of two patients. A summary of the clinical rebound sequences in each of the 64 patients in this survey is given in Figures 4 to 6. The 100 post-therapeutic clinical rebounds of these 64 patients have been analyzed in these figures according to their clinical features and the associated use of suppressive therapy. The results are described below:

1. *Patients with no valvular involvement* (Fig. 4)

In 18 patients who originally had no valvular involvement, the clinical rebounds produced fever, tachycardia, or joint symptoms, but no evidence of carditis. Thus, recurrent rheumatic activity *in the same attack* did not bring valvular involvement to patients who were initially free of it, and the results are consistent with preceding data which indicate that this group of patients usually remains free of heart disease *even in a completely new attack of rheumatic fever.*[8] Of the clinical rebounds in this group, 11 were untreated and all subsided spontaneously without any additional effects. Of the primary clinical rebounds, 7 were treated with suppressive agents, and in 5, when the treatment was stopped, there was a subsequent clinical rebound, without carditis. The secondary rebounds were then left untreated and subsided spontaneously.

2. *Patients with valvular involvement, without significant cardiomegaly* (Fig. 5)

The first clinical rebound brought evidence of new carditis to 8 of the 28 patients with clinical rebounds who initially had valvular involvement without significant cardiomegaly. In each case, the carditis was manifested by a diastolic murmur which was not previously present. Of these 8 patients, 4 were untreated and the other manifestations of the rebound subsided spontaneously; in 3, the new murmur disappeared before the end of the convalescent period.* The other 4 "carditic" rebounds were treated and 3 had a subsequent clinical rebound, (1 with carditis, 2 without) after therapy was stopped again. Two of the secondary rebounds were then left untreated and

* The one patient in whom the new murmur persisted was found to be free of it when re-examined two years later.

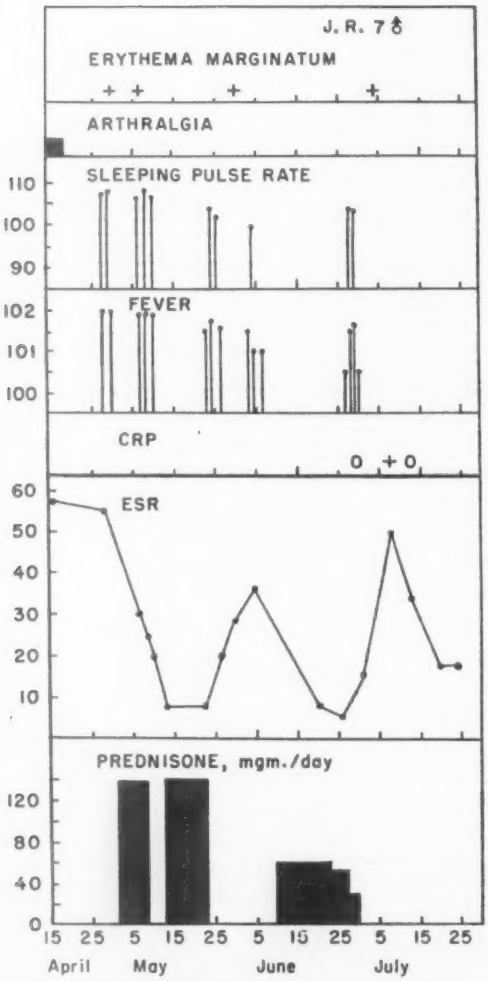


FIG. 3. *Recurrent episodes of fever and erythema marginatum after repeated courses of steroid treatment.* In this 7-year-old boy's first attack of rheumatic fever, the arthralgias disappeared before hospitalization. On admission he had the apical systolic murmur of mitral regurgitation, slight enlargement of the left atrium, fever, and erythema marginatum. The murmur and slight cardiomegaly persisted thereafter. The fever and erythema marginatum subsided after one week of prednisone, returned when it was stopped, subsided when it was resumed, returned when it was again stopped and then subsided and reappeared once more following a third course of prednisone therapy. They then subsided spontaneously.

all of their clinical features subsided spontaneously. The other was treated, and cessation of treatment was followed by a tertiary rebound.

The other 20 primary clinical rebounds in this group consisted of fever alone in 7, and of joint features and/or nonfebrile tachycardia in 13. Of the 8 noncardiac primary rebounds which were left untreated, all but 2 subsided without recurrence. One of these patients had a spontaneous secondary clin-

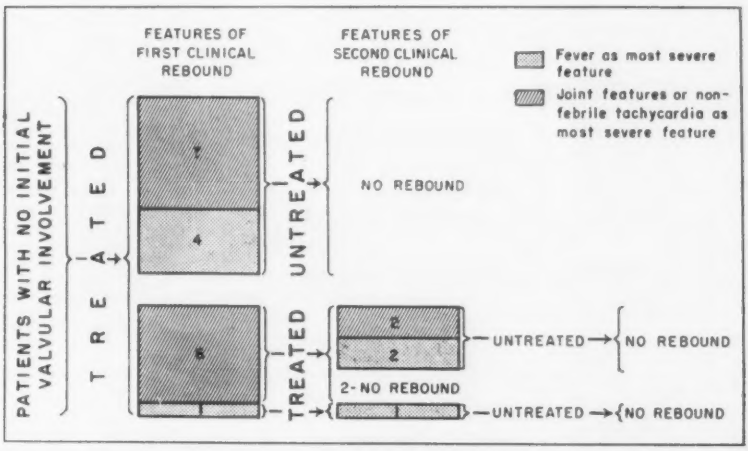


FIG. 4. *Sequence and nature of events before and after clinical rebounds in 18 patients with no initial valvular involvement.* This figure and Figs. 5 and 6 follow a similar arrangement. The blocks at the left represent a group of patients, with cardiac status as indicated, who had a clinical rebound when their first course of anti-rheumatic treatment was stopped. The arrows pointing to the right indicate the subsequent events in *each* patient, with or without additional clinical rebounds, until rheumatic activity ended. The numbers refer to the number of patients in each category. The patients who were "treated" received steroids and/or salicylates. The shading of the blocks indicates the most severe features of the rebounds, as shown in the associated legend of each figure. For further details, see text.

ical rebound manifested again by joint symptoms, while the other (who previously had had only fever) developed a spontaneous secondary rebound, manifested by a new diastolic murmur. In this patient, the second rebound was treated, and the new murmur and other manifestations then disappeared. In the first patient, all abnormalities subsided without further treatment.

Suppressive treatment was given to 12 of the clinical rebounds in whom there was no evidence of provoked carditis. The cessation of treatment was followed by another rebound in 7 patients and of these secondary rebounds, 2 were treated, both of whom had a third rebound when treatment was

stopped. The tertiary rebounds were then left untreated and subsided spontaneously. Of the 5 secondary rebounds which were not treated, 4 subsided spontaneously; arthritis reappeared as a tertiary rebound in 1 patient who had had persistent chorea in the interval between rebounds.

It should be noted that the appearance of new murmurs was the only major feature of carditis in the clinical rebounds of this group of patients.

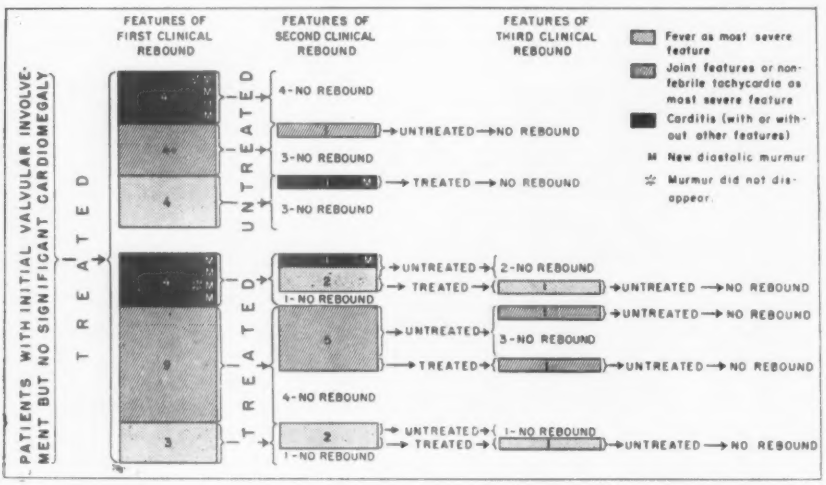


FIG. 5. *Sequence and nature of events before and after clinical rebounds in 28 patients who initially had valvular involvement but no significant cardiac enlargement.* (See further description of Fig. 4.)

Congestive failure or cardiac enlargement did not occur. Of the 10 murmurs which appeared at the time of the rebounds, all but 2 disappeared after several months, and the disappearance seemed to be unrelated to the presence or absence of additional therapy during the rebounds.

3. *Patients with valvular involvement, with significant cardiomegaly* (Fig. 6)

Of the 18 clinical rebounds in this group, 5 had new carditis, manifested by congestive failure in 2, by new diastolic murmurs in another 2, and by pericarditis in the last. All of the carditic rebounds and 5 noncarditic rebounds were treated; all 10 of the treated rebounds were followed by a secondary clinical rebound when the treatment was discontinued. In the

group who had carditis with the first rebound, the second rebound renewed the congestive failure in the two patients who had it previously, and brought pericarditis to a patient whose previous newly acquired murmur had disappeared in the interval between the first and second rebound. Two others developed only fever. Both of the febrile rebounds were treated and both were followed by another rebound; the tertiary rebounds were untreated

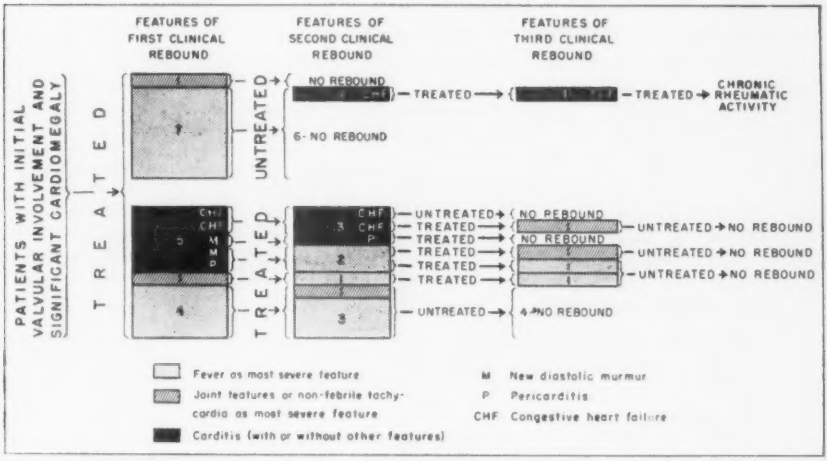


FIG. 6. *Sequence and nature of events before and after clinical rebounds in 18 patients with valvular involvement and significant cardiac enlargement.* (See further description of Fig. 4.)

and subsided spontaneously. One of the congestive failure rebounds was treated and was followed by another rebound, manifested by arthritis. The other congestive failure rebound was untreated and subsided spontaneously.

A group of 8 clinical rebounds were left untreated. These rebounds manifested by fever alone in 7 and by arthritis in 1. All but one subsided without recurrence. In one patient, a second rebound occurred spontaneously and included increased cardiomegaly with congestive failure. Its treatment at this time was followed by a tertiary rebound which was treated, followed by another rebound. This patient had unusually prolonged rheumatic activity, of the type described in a separate survey of "chronic" rheumatic fever.[28]

Figures 4 to 6 illustrate that clinical rebounds did not bring carditis to those patients who did not have it previously; in the patients with previous

carditis, they brought significant cardiac enlargement or congestive failure only to those who had had it previously. A major point of these figures is better demonstrated in Table 1 which shows the occurrence of subsequent rebounds in relation to the type of each clinical rebound and its management. In patients whose rebound consisted only of fever or febrile tachycardia, additional rebounds occurred in 11 of the 12 patients whose previous

TABLE 1. OCCURRENCE OF SUBSEQUENT CLINICAL REBOUNDS IN RELATION TO TYPES OF CLINICAL REBOUND AND THEIR MANAGEMENT

| | *Anti-inflammatory treatment given to clinical rebound* | | *No anti-inflammatory treatment given to clinical rebound* | |
|---|---|---|---|---|
| *Classification of clinical rebounds according to most severe manifestations* | *Number of episodes* | *Number followed by subsequent clinical rebounds* | *Number of episodes* | *Number followed by subsequent clinical rebounds* |
| Fever or febrile tachycardia | 12 | 11 | 27 | 2 |
| Joint symptoms or signs, and/or nonfebrile tachycardia, and/or erythema marginatum | 17 | 11 | 24 | 2 |
| New diastolic murmurs | 7 | 5 | 5 | 0 |
| Pericarditis and/or congestive heart failure | 7 | 6 | 1 | 0 |
| Total | 43 | 33 | 57 | 4 |

rebound was treated, but only in 2 of the 27 whose previous rebounds were left untreated. In patients whose rebound consisted only of joint abnormalities, nonfebrile tachycardia, or erythema marginatum, with or without fever, subsequent rebounds occurred in 11 of the 17 patients whose earlier rebounds were treated, but only in 2 of the 24 who were untreated. In patients with new diastolic murmurs, subsequent rebounds appeared in 5 of the 7 who were treated, and in none of 5 who were not treated. When the rebound included more severe cardiac manifestations, additional rebounds occurred in 6 of the 7 patients who were treated but did not occur in the 1 patient who was left untreated.

These results demonstrate that most clinical rebounds subside without additional therapy and without additional complications. Two possible

exceptions may be noted: (*i*) patients with severe carditis during a primary rebound are usually treated immediately and the untreated sequelae of this complication are therefore uncertain; (*ii*) patients with "chronic" rheumatic activity, discussed separately,[28] show a truly polycyclic course in which relapses often occur without relationship to preceding therapy. The presence of "chronic" rheumatic activity may be suspected when a clinical

TABLE 2. OCCURRENCE OF SUBSEQUENT CLINICAL REBOUNDS IN RELATION TO TYPES OF CLINICAL REBOUND AND AGENT USED FOR THEIR TREATMENT

| *Classification of clinical rebounds according to most severe manifestation* | *Agent used to treat rebounds** | | |
|---|---|---|---|
| | *Salicylates* | *Steroids* | *Combined therapy* |
| Fever or febrile tachycardia | 2/3 | 9/9 | 0/0 |
| Joint symptoms or signs and/or nonfebrile tachycardia and/or erythema marginatum | 5/11 | 6/6 | 0/0 |
| New diastolic murmurs, pericarditis, or congestive heart failure | 4/5 | 4/5 | 3/4 |
| Total | 11/19 | 19/20 | 3/4 |

* In each instance, the denominator represents the number of patients whose clinical rebound was treated with this agent; the numerator represents the number in whom another clinical rebound occurred when treatment was reduced or discontinued.

rebound occurs without a temporal relation to preceding therapy or when it fails to resolve spontaneously.

*Agent of treatment for clinical rebounds*

Table 2 shows the types of clinical rebounds which were treated, the anti-inflammatory agents used, and the occurrence of subsequent rebounds when these agents were reduced or discontinued. In the rebounds which did not produce new carditis, subsequent rebounds occurred in all of the 15 patients treated with steroids and in only 7 of the 14 treated with salicylates. It is not likely that this could occur by chance alone ($X^2 = 7.3$, $p < 0.01$).

In the group who had carditis with their clinical rebounds, none of the three therapeutic regimens was able to prevent or affect the recurrence of subsequent clinical rebounds. They re-appeared after 4 of the 5 episodes treated with salicylates, 4 of the 5 treated with steroids, and 3 of the 4 which received combined therapy.

*Duration of rheumatic "rebound" activity*

The duration of the new rheumatic inflammation which occurred with clinical rebounds is best measured in those which were not treated. Table 3 shows how long the acute clinical and laboratory abnormalities persisted in the 31 primary clinical rebounds which did not receive further therapy. (It should be noted that none of the rebounds in this untreated group included congestive failure. The most severe cardiac features were new diastolic murmurs or nonfebrile tachycardia.) In contrast to the relatively short duration of the clinical features in these rebounds, the associated laboratory abnormalities lasted for a month or more in a majority of patients. The clinical abnormalities subsided for 21 patients in less than 1 week and for 8 patients in less than 2 weeks. In the latter group, the manifestations which lasted longer than a week were fever, tachycardia, or arthralgias. In 2 of the patients with previous valvular involvement, nonfebrile tachycardia persisted for 2 to 3 weeks and, in each case, a spontaneous clinical rebound occurred later, and the patients showed "chronic" rheumatic activity.

These results suggest that most clinical rebounds, with the exception of those involving congestive failure, will spontaneously subside within two weeks even though their laboratory abnormalities may last much longer. If the clinical features persist for more than 2 weeks, the presence of "chronic" rheumatic activity is likely.

*Prevention of clinical rebounds*

The previous study[6] demonstrated that clinical rebounds did not occur in patients who were either untreated or who received a long course (more than 8 weeks) of salicylates alone. These two therapeutic approaches are generally used, however, only in patients with little or no cardiac involvement. Many physicians will continue to use steroids for patients with severe carditis, and with few exceptions[36] will maintain suppressive therapy for at least 8 weeks. Previous work[2, 6, 13, 15] has shown that steroid therapy, given alone for this length of time, is frequently followed by clinical rebounds regardless of the methods used to reduce and stop the medication.

A number of therapeutic designs have attempted to prevent these post-steroid rebounds. Most designs have involved treatment with a combination of steroids and salicylates in which steroids are started, salicylates are added at some point, and salicylates are continued after the steroids are stopped. The differences in various programs depend upon the time at which the salicylates are added. One plan[5] starts the two drugs simultaneously, another adds salicylate after a week of steroids,[13] a third begins salicylates when the sedimentation rate has reached normal values on steroids.[15]

TABLE 3. DURATION OF ACUTE ABNORMALITIES IN UNTREATED CLINICAL REBOUNDS

| *Original cardiac status of patient* | *Type of abnormality in clinical rebound* | *Duration of abnormality* | | | | |
|---|---|---|---|---|---|---|
| | | *1 week or less* | *1-2 weeks* | *2-3 weeks* | *3-4 weeks* | *More than 4 weeks* |
| No valvular involvement | Clinical | 8 | 3 | | | |
| | Laboratory | 1 | 3 | 2 | 2 | 3 |
| Valvular involvement | Clinical | 13 | 5 | 2 | | |
| | Laboratory | 1 | 2 | 4 | 6 | 7 |

TABLE 4. SEQUELAE OF THERAPY IN TWO TYPES OF COMBINED TREATMENT REGIMENS IN ACUTE RHEUMATIC FEVER

| *Initial clinical status* | *"Simultaneous" therapy* (*Steroids* / *Salicylates*) | | | | *"Overlap" therapy* (*Steroids* / *Salicylates*) | | | |
|---|---|---|---|---|---|---|---|---|
| | *No. of patients* | *Number of patients with:* | | | *No. of patients* | *Number of patients with:* | | |
| | | *No rebounds* | *Laboratory rebounds* | *Clinical rebounds* | | *No rebounds* | *Laboratory rebounds* | *Clinical rebounds* |
| No valvular involvement | 9 | 3 | 4 | 2 | 8 | 2 | 6 | 0 |
| Valvular involvement without significant cardiomegaly | 9 | 5 | 3 | 1 | 8 | 2 | 6 | 0 |
| Valvular involvement with significant cardiomegaly | 7 | 4 | 1 | 2 | 5 | 2 | 1 | 2 |
| Total | 25 | 12 | 8 | 5 | 21 | 6 | 13 | 2 |

The present data and those acquired in the previous[8] study may be used to compare the effectiveness of these regimens. Table 4 shows the results of two types of combined treatment programs. In both programs, the salicylates were continued after steroids were stopped. In the "simultaneous" regimen, the two drugs were started at the same time. In this group, steroids were maintained for an average of 30 days and salicylate for 60 days. Of the 25

TABLE 5. INCIDENCE OF PRIMARY CLINICAL REBOUNDS IN RELATION TO INITIAL CLINICAL STATUS: COMPARISON OF LONG COURSES (EIGHT WEEKS OR MORE) OF STEROID TREATMENT AND COMBINED THERAPY

| | *Steroid treatment alone* | | | *Combined therapy** | | |
|---|---|---|---|---|---|---|
| *Initial clinical status* | *No. of patients* | *No. of clinical rebounds* | *Percentage* | *No. of patients* | *No. of clinical rebounds* | *Percentage* |
| No valvular involvement | 4 | 2 | 50% | 17 | 2 | 12% |
| Valvular involvement without significant cardiac enlargement | 15 | 9 | 60% | 17 | 1 | 6% |
| Valvular involvement with significant cardiac enlargement | 11 | 7 | 64% | 12 | 4 | 33% |
| Total | 30 | 18 | 60% | 46 | 7 | 15% |

* The numbers used here represent the *sum* of results obtained in "simultaneous" and "overlap" combined therapy regimens, as cited in Table 4.

patients, 8 had a laboratory rebound and 5 had a clinical rebound, manifested in 3 by joint manifestations and in 2 by fever; none had new cardiac involvement. The "overlap" regimen was tested as described above, and in this program salicylates were started only when the reduction of steroids began. For these patients, the length of steroid tapering, and thus of the "overlap" period, averaged two weeks. The average duration of steroid treatment in this group was 50 days, and salicylates were maintained for an additional 16 days after steroids were stopped. Of the 21 patients who received this type of therapy, 13 had laboratory rebounds. Clinical rebounds occurred only in 2 patients, both of whom had marked cardiac enlargement and who later demonstrated persistent "chronic" activity.

These results suggest that "overlap" therapy is as good as "simultaneous" combined therapy in preventing clinical rebounds. The data also suggest that overlap therapy may even be superior, particularly in patients with slight or no cardiac involvement, but the numbers are not large enough for statistical significance.

The therapy in both of these combined treatment regimens was maintained for at least 8 weeks and their assembled results are compared in Table 5 against the data obtained previously[6] when steroids were used alone for this length of time. In each of the three cardiac categories, the percentage of clinical rebounds was lower after combined therapy than after a similar duration of steroids alone. In the total results this reduction is statistically significant ($X^2 = 14.5$, $p < 0.01$). It should be noted that the data for "combined therapy" in the present tables differ somewhat from those presented earlier.[6] The difference arises because the previous data represented total results obtained with a *variety* of "combined" therapy programs while the present data are restricted to the results of two specifically designed programs.

It would seem that when planning a long course of suppressive therapy, especially if steroids are to be used, the incidence of subsequent clinical rebounds will be reduced by adding salicylates to the program, either initially or when the reduction of steroids begins, and by maintaining salicylates alone for several weeks after steroids have been stopped.

## DISCUSSION

The management and prevention of post-therapeutic clinical rebounds in acute rheumatic fever has been the subject of numerous, and often conflicting, recommendations. Some of the problems arise, as noted previously, from the use of different criteria for definition of a rebound, from the lack of differentiation between rebounds which included clinical abnormalities and rebounds whose abnormalities were only in laboratory tests, and from the failure of most published reports to classify the preceding clinical features and to correlate them with the manifestations of the rebound. The present investigations have shown that the rebound comprises a wide spectrum of laboratory and clinical signs ranging from a slight, transient elevation of the sedimentation rate in a patient with no cardiac involvement to severe clinical manifestations with new and life-threatening cardiac damage in patients with extensive valvular disease and marked cardiac enlargement. Obviously the prevention, prognosis, and management of rebounds requires a consideration of the severity of the rheumatic attack and of the severity of the manifestations of the rebounds.

*Prevention of rebounds*

For patients without valvular involvement during the acute attack, it has been shown that clinical rebounds can be prevented by withholding anti-inflammatory therapy, by giving "overlap" combined therapy or by using salicylates alone for more than 8 weeks. Since the rebounds in this group are so benign, the use of long-duration salicylate therapy does not seem warranted, and a short course may be attempted although a rebound may follow it.

For patients with valvular involvement, many physicians are reluctant to withhold anti-inflammatory therapy and usually want to maintain it for at least 8 weeks. When treatment is given for this length of time, rebounds are rare after salicylates alone but occur frequently after steroids. Physicians who feel that steroids are preferable to salicylates for the routine treatment of this group of patients should add salicylates to the regimen in order to minimize the post-steroid rebounds. "Overlap" combined therapy is as effective as "simultaneous" combined therapy and offers two minor advantages: (*i*) the salicylate portion can be appended flexibly, according to the clinical course, at the time when the steroids have totally suppressed the rheumatic activity and a graded progressive reduction in their dosage begins and (*ii*) salicylate toxicity may be less likely to occur when the drug is used for a shorter period.

*Management of rebounds*

For the management of rebounds, the available suggestions range from those which urge that almost all rebounds be treated immediately[8, 21] to those which recommend resumption of treatment only when the clinical features are severe and threatening[9, 18] and, finally, to those which propose an observation period to allow spontaneous resolution; treatment is then resumed if the rebound fails to subside in an appropriate period of time.[11, 17] The permitted length of time varies from 24 hours to 3 weeks.

The present and previous[6] results indicate that a laboratory rebound is a minor, self-limited event which will not affect the patient's clinical status, which does not need further treatment, and which may even be made worse by further treatment. Additional suppressive therapy will also perform no useful service for those laboratory rebounds which recur spontaneously or which do not subside in several weeks, since this treatment will only prolong the period of invalidism and since many of the laboratory rebounds represent benign elevations in sedimentation rate which may persist for months or years[20] without altering the patient's clinical condition.

The management of clinical rebounds needs more individualized attention. Therapy for the rebound has never been known to eliminate any pathological features which existed *before* the rebound and can be expected only to remove those which were brought by the rebound. The present data demonstrate that many of the clinical features of rebounds will subside spontaneously, like their laboratory counterparts, and that additional anti-inflammatory treatment is often followed by repeated secondary or even tertiary rebounds. If the hypothesis is correct that many clinical rebounds are due to the appearance of previously suppressed rheumatic inflammation, it would seem reasonable to allow the inflammation to be dispersed without suppression when its immediate effects are not life-threatening.

a) *Patients with no valvular involvement initially*

These patients are not likely to develop future heart disease[4] and their clinical rebounds quickly subside spontaneously, do not recur, and do not produce cardiac damage. Suppressive therapy for these rebounds will often be followed by additional rebounds and an unnecessary prolongation of the total duration of rheumatic activity.

b) *Patients with valvular involvement but without significant cardiac enlargement*

In this group of patients, the clinical rebounds may include new cardiac manifestations; these consisted, in the present survey, only of new diastolic murmurs without any more severe events. The noncardiac features—fever, arthritis or arthralgia—almost always subsided spontaneously. Additional treatment for them conferred no benefits and carried the hazard of subsequent rebounds. Thus, the main decision regarding the management of clinical rebounds in this group has to do with the therapy for a newly appeared murmur. In the present survey, there were 12 such murmurs in the rebound period, and all but 10 vanished by the end of convalescence, whether or not they had been treated. The high incidence with which these "rebound" murmurs disappear, irrespective of treatment, suggests that they do not necessarily indicate new "valvulitis" and that they may be due to temporary alterations, to fever or tachycardia, and hence hemodynamic flow through an already damaged valve. Suppressive therapy for these physical factors may conceivably minimize further damage. If treatment is used, salicylates would be preferable to steroids because they are less likely to be followed by subsequent rebounds.

c) *Patients with significant initial cardiac enlargement**

This group of patients was the only one in which clinical rebounds brought severe or life-threatening cardiac features: pericarditis, increasing cardiomegaly, or congestive heart failure. For these complications, the resumption of anti-inflammatory therapy seems mandatory even though its cessation will be followed by another rebound. The hope is that the subsequent rebounds will be milder, and that the rheumatic inflammation will ultimately consume itself without further cardiac damage. None of the suppressive agents seems to be superior for this purpose in this group.

SUMMARY

A rebound in acute rheumatic fever was defined as the reappearance of rheumatic activity after it had originally disappeared under or without suppressive treatment and in the absence of an intercurrent streptococcal infection. A laboratory rebound, manifested by abnormalities only in acute phase reactants of the blood, occurred frequently, sometimes recurred after its initial cessation, had no clinical effects, and always subsided spontaneously.

Clinical rebounds, manifested by clinical as well as laboratory abnormalities, appeared in 64 of 415 consecutive admissions of children and adolescents with acute rheumatic fever. A total of 100 clinical rebounds occurred. Their severity and possible prevention were directly dependent upon the original manifestations of the rheumatic attack. The results of their management and prognosis depended upon the severity of the initial attack and the features of the individual rebounds.

In patients who did not have valvular involvement initially, the clinical rebounds did not bring cardiac manifestations. In patients with valvular involvement but without significant cardiac enlargement, the rebounds, at their worst, brought only new murmurs but no more severe cardiac effects. In both these groups, the manifestations of the rebounds almost always subsided spontaneously within two weeks. When treated with suppressive agents, the rebounds were often followed by additional rebounds when the agents were discontinued. A subsequent rebound after anti-inflammatory treatment of a previous rebound was less likely after salicylates than after steroids. In both these clinical groups, the rebounds could be prevented by

* The decision that the heart is enlarged is a subtle matter. Many "slightly enlarged" hearts can be "cured" by use of an appropriately wide range of normal limits or by the realization that the child's or adolescent's cardiac silhouette may normally change considerably with different phases of the cardiac or respiratory cycle.

not using anti-inflammatory agents for the initial attack or by treating the initial attack with salicylates alone for more than 8 weeks. In patients of these two groups who were initially treated with steroids, the incidence and severity of rebounds could be markedly reduced or prevented by adding salicylates at the time of inception or reduction of steroid treatment and by continuing salicylates for several weeks after steroids were stopped.

In a third group of patients, who initially had significant cardiac enlargement, the clinical rebounds could bring pericarditis, increased cardiomegaly, or congestive failure, in addition to other noncardiac features. When confined to noncardiac features, these rebounds also usually subsided spontaneously and frequently recurred if treated. The severe cardiac features of primary rebounds were all treated with suppressive agents, whose cessation was always followed by secondary clinical rebounds. No specific therapeutic approach to the primary rebound seemed to reduce the occurrence of secondary rebounds in this group. The use of specific kinds of combined steroid-salicylate therapy for the *initial attack* in these patients could reduce but not completely prevent the incidence of post-therapeutic primary clinical rebounds.

"Chronic" rheumatic activity, of unusually prolonged duration, appeared in a small percentage of patients and was characterized by clinical rebounds which did not subside spontaneously and which occurred without a direct temporal relationship to preceding suppressive treatment.

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ABNER GOLDEN*
PHILIP K. BONDY**
ROBERT W. CHAMBERS†
C. LEONARD GEIGER‡

*Department of Pathology, Emory University School of Medicine, and the Department of Internal Medicine, Yale University School of Medicine*

# ADENOHYPOPHYSEAL CHANGES IN PATIENTS DYING OF NEOPLASTIC DISEASE¶

We recently described cytological findings in the adenohypophyses of a group of patients dying in uremia of acute renal tubular necrosis.[4] Dramatic changes in the acidophilic cells were interpreted as the morphological expression of altered pituitary function in response to malnutrition. Changes in the basophils were thought to result from both malnutrition and renal excretory failure. The patients had all been well until the onset of uremia and were maintained during their illness on a limited caloric intake derived solely from carbohydrates.

These observations suggested a more extensive investigation of the effects of nutritional status on pituitary morphology. The present report describes the adenohypophyseal findings in a group of thirteen patients dying of neoplastic disease. A smaller group of patients dying of non-neoplastic chronic disease associated with severe malnutrition is included for comparison. The study strongly suggests that certain alterations in pituitary cytology can be correlated with nutrition during the terminal period of life.

## METHODS

*Adenohypophyses.* The method of study of the adenohypophyses has been described in detail elsewhere.[3] The glands were fixed in buffered 10 per cent formalin, USP. They were cut so as to first remove a 1 mm. mid-saggital section including the pituitary stalk; the remaining portions were bisected, one in the equatorial, the other in the mid-coronal plane. Paraffin sections of each plane were stained by multiple methods, Pearse trichrome PAS[7] sections being utilized for quantitative study.

* Professor of Pathology, Emory University School of Medicine.
** Associate Professor of Medicine, Yale University School of Medicine.
† Senior Medical Student, Emory University School of Medicine.
‡ Assistant Resident in Pathology, Emory University School of Medicine.
¶ This work was aided by a grant from the National Institute of Arthritis and Metabolic Disease, United States Public Health Service, Department of Health, Education, and Welfare (A-1719). The technical assistance of Mrs. Elizabeth Fortson is gratefully acknowledged.
*Received for publication 27 June 1960.*

Differential cell counts were performed by examining contiguous "high-dry" microscopic fields so as to cover an entire histological section.

Cells were classified as follows:

*"Storage basophils."* Cells with small nuclei and cytoplasm tightly packed with PAS positive granules. These are the "basophils" of most authors and the "maximal mucoid" cells of Pearse.[8]

*"Secretory basophils."* Large cells with generally prominent nuclei and delicate cytoplasmic stippling by fine, predominantly PAS positive granules ("intermediate and punctate mucoid" cells of Pearse[8]; "amphophils" of Russfield[15]).

*"Storage acidophils."* Cells with small nuclei and dense orangophilic cytoplasmic granulation ("acidophils" of most authors).

*"Secretory acidophils."* Large cells with prominent, frequently hyperchromatic and pleomorphic nuclei and cytoplasm containing delicate, predominantly orangophilic granules. These cells would probably be included as "amphophils" by Russfield.[15]

*"Hypersecretory cells."* Cells with very large hyperchromatic or vesicular nuclei and variable cytoplasmic mass containing only occasional PAS positive or orangophilic granules ("hypertrophic amphophils" of Russfield[15]).

*"Chromophobes."* Cells with small nuclei and scant agranular cytoplasm.

*Other tissues.* Sections of other organs including the endocrine glands were fixed in Zenker's fluid with 5 per cent glacial acetic acid, embedded in paraffin and stained with phloxine and hematoxylin.

*Evaluation of terminal diet.* Dietary intake during the terminal seven days of life was arbitrarily designated as "good," "fair," "poor," or "nil" on the basis of estimated average daily intake for this period: *good;* more than 1,000 calories including over 40 gm. protein, *fair;* 500 to 1,000 calories including 15 to 40 gm. protein, *poor;* less than 500 calories and less than 15 gm. protein, *nil;* few, if any calories and no protein.

## RESULTS

### *Patients dying of neoplastic disease*

*Clinical observations.* The significant clinical data are recorded in Table 1. With the exception of patient 1 who died of bilateral pulmonary alveolar cell carcinoma, all patients had regional or distant metastases from their primary tumors. The two patients dying of leukemia evidenced multiple organ involvement.

The duration of illness varied from two months to ten years. Significant weight loss was indicated in those patients from whom a reliable history was available and most patients were described as poorly nourished, if not cachectic.

Dietary intake during the terminal seven days of life was characterized as "good" in three patients who ate well up to the time of death, "fair" in two, and "poor" in seven. Patient 13, the only patient not hospitalized during the terminal seven days, refused all nourishment and received water only.

TABLE 1. CLINICAL AND MORPHOLOGICAL DATA ON PATIENTS DYING OF NEOPLASTIC DISEASE

The normal values for pituitary cytological differential counts were derived from the study of healthy individuals on normal diets who were victims of sudden death.[2,3] Hormonal therapy, when received, is indicated by symbols. Data on weight loss are based primarily on clinical history. The adrenal weights represent the combined weight of both glands.

| No. | AGE SEX | DIAGNOSIS AND DURATION | TERMINAL HEIGHT | HEIGHT LOSS | DIETARY INTAKE TERMINAL 7 DAYS | PITUITARY CYTOLOGY (PERCENTAGES) ACIDOPHILS STORAGE | ACIDOPHILS SECRETORY | HYPER-SECRETORY CELLS | BASOPHILS STORAGE | BASOPHILS SECRETORY | CHROMOPHOBES | ADRENALS | THYROID | GONADS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 59-F | ALVEOLAR CA, 1 YR.* | 38 K 162 cm | 14 K | GOOD | 57.7 | 5.1 | 3.3 | 14.6 | 3.8 | 15.5 | 9.0 gm NORMAL | 12.0 gm SLIGHT INVOLUTION | ATROPHY ▷ |
| 2. | 52-F | OVARIAN CA, 1 YR. | 55 K 177 cm | - | GOOD | 57.5 | 5.7 | 3.0 | 10.3 | 8.7 | 14.8 | 12.5 gm LIPOID DEPLET'N | NORMAL | DESTROYED BY TUMOR |
| 3. | 58-M | BRONCHOGENIC CA, 6 MOS.* | 60 K 183 cm | 4 K | GOOD | 57.5 | 10.7 | 5.1 | 8.0 | 4.2 | 14.5 | 11.6 gm LIPOID DEPLET'N | 17.0 gm INVOLUTION | SEVERE ATROPHY |
| 4. | 68-F | MYELOBLASTIC LEUKEMIA 2 MOS.* | 60 K 162 cm | 5 K | FAIR | 45.7 | 8.7 | 6.6 | 16.9 | 12.7 | 9.4 | 12.5 gm LIPOID DEPLET'N | 13.0 gm INVOLUTION, FIBROSIS | ATROPHY ▷ |
| 5. | 41-F | MYELOBLASTIC LEUKEMIA 1 YR.* | 52 K 168 cm | 2 K | FAIR | 59.5 | 10.4 | 5.3 | 9.6 | 5.2 | 10.0 | 14.4 gm CORTICAL ATROPHY | 13.0 gm INVOLUTION, FIBROSIS | DESTROYED BY TUMOR |
| 6. | 46-M | BRONCHOGENIC CA, 6 MOS. | 50 K 174 cm | - | POOR | 47.7 | 18.2 | 9.7 | 11.6 | 4.7 | 8.1 | 21.9 gm CORTICAL ADENOMA NORMAL LIPOID | 10.7 gm NORMAL | MODERATE ATROPHY |
| 7. | 61-M | BRONCHOGENIC CA, 6 MOS. | 68 K 167 cm | 9 K | POOR | 37.9 | 22.1 | 8.1 | 21.9 | 2.8 | 7.2 | 26.0 gm METAS, LIPOID DEPLET'N | 17.0 gm MOD. INVOLUTION | MARKED ATROPHY |
| 8. | 54-M | BRONCHOGENIC CA, 6 MOS.* | 68 K 180 cm | - | POOR | 35.6 | 26.3 | 7.8 | 15.5 | 8.3 | 6.5 | 11.5 gm CORTICAL ATROPHY | 12.0 gm MOD. INVOLUTION | MARKED ATROPHY |
| 9. | 50-M | CA. OF ESOPHAGUS 2 MOS. * | 47 K 170 cm | 6 K | POOR | 50.4 | 17.2 | 7.7 | 10.2 | 9.2 | 5.3 | 13.0 gm SLIGHT LIPOID DEPLET'N | 16.0 gm SLIGHT INVOLUTION | DECREASED SPERMATOGENESIS |
| 10. | 69-F | CA. OF BREAST 4 YRS.ø | 30 K 145 cm | 9 K | POOR | 55.4 | 13.9 | 4.5 | 9.3 | 8.5 | 8.4 | 13.0 gm SLIGHT LIPOID DEPLET'N | 7.0 gm SLIGHT INVOLUTION | DECREASED SPERMATOGENESIS |
| 11. | 53-F | CA. OF PANCREAS 4 YRS.ø | 64 K 163 cm | - | POOR | 37.5 | 20.7 | 10.3 | 19.4 | 5.1 | 7.0 | 13.9 gm MARKED LIPOID DEPLET'N | 11.1 gm SEVERE ATROPHY | ABSENT |
| 12. | 62-F | TUBAL CA 6 MOS. | 50 K 161 cm | 23 K | POOR | 36.7 | 21.1 | 11.5 | 15.6 | 3.9 | 11.2 | 19.4 gm MARKED LIPOID DEPLET'N | 11.0 gm MOD. INVOLUTION | ATROPHY ▷ |
| 13. | 71-F | CA. OF BREAST 10 YRS.Σ | 30 K 151 cm | - | NIL | 10.9 | 50.6 | 23.9 | 8.1 | 2.7 | 3.8 | 13.0 gm MODERATE LIPOID DEPLET'N | 16.5 gm NORMAL | ATROPHY ▷ |
| NORMAL RANGE | | | | | | 44.3 TO 60.0 | 1.2 TO 2.8 | 0.0 TO 0.3 | 8.6 TO 16.8 | 6.5 TO 10.1 | 21.4 TO 34.7 | | | |

* = ADRENAL STEROID ** = ACTH TREATMENT ø = ESTROGEN Σ = ANDROGEN ▷ = POSTMENOPAUSAL

In several instances, patients who had lost considerable weight and were poorly nourished ate surprisingly well during their terminal period in the hospital.

None of the patients was uremic at the time of death and none was found to have renal metastases or other significant renal disease. Several patients received hormone preparations as part of their therapy (see Table 1).

*Morphological observations.* The adenohypophyses of all these patients showed striking changes. These consisted of increased proportions of secretory acidophils and hypersecretory cells.

Table 1 shows the results of differential cell counts performed on the equatorial sections of the glands. (The normal values recorded were derived from the study of hypophyses of healthy individuals on normal diets who were victims of sudden death.[7, 8]) Secretory acidophils, which normally represent 1 to 3 per cent of all cells, here comprised 5.1 to 50.6 per cent. Hypersecretory cells, rarely seen in normal glands, accounted for 3.0 to 23.9 per cent of all cells, the increases paralleling those of the secretory acidophils. Many transition forms between secretory acidophils and hypersecretory cells were noted. The prominence of secretory acidophils and hypersecretory cells appeared to be at the expense of both storage acidophils and chromophobes.

The basophilic elements did not show consistent alterations. Storage basophils were increased above normal in several instances and displayed Crooke's hyaline change in those patients receiving adrenal steroid therapy. A decreased percentage of secretory basophils was noted in eight glands.

The findings in the adrenal glands, thyroids, and gonads are shown in the table. The adrenal glands were within normal weight range with the exception of Case 7, where metastatic disease was present, Case 6, where one gland contained a cortical adenoma, and Case 12, where an increased weight was unexplained. Most of the glands showed varying degrees of lipoid depletion. The thyroid glands were characterized histologically as normal in three instances, involutional in eight, and atrophic in two. The gonads showed decreased activity or atrophy in all patients. Five of the female patients were post-menopausal.

Fatty metamorphosis of the liver was noted in most instances, but its severity could not be correlated with other morphological observations.

*Patients dying of non-neoplastic chronic disease*

The findings in the patients dying of non-neoplastic chronic disease are shown in Table 2. All gave a history of significant weight loss and were poorly nourished. (The weight recorded for patient 18 reflects in part a massive edema). The terminal dietary intake of five patients was characterized as "poor." Patient 19 received only intravenous fluids with some carbohydrate added.

Two patients died in uremia of chronic glomerulonephritis, two of chronic ulcerative colitis, one 14 months after extensive gastro-intestinal and partial pancreatic resection for what proved to be a benign gastric ulcer,

and one of an unclassified systemic granulomatous disease. Two patients received adrenocorticotropin therapy; two, adrenal steroids.

The adenohypophyses of these patients showed increased proportions of secretory acidophils (14.9 to 45.4 per cent) and hypersecretory cells (5.8 to 19.4 per cent) comparable to the findings in the patients dying of neoplastic disease. Secretory basophils were reduced in percentage in all glands in this group. The findings in the other endocrine organs were similar to those in the neoplastic group.

TABLE 2. CLINICAL AND MORPHOLOGICAL DATA ON PATIENTS DYING OF NONNEOPLASTIC CHRONIC DISEASE

| No. | AGE SEX | DIAGNOSIS AND DURATION | TERMINAL WEIGHT | WEIGHT LOSS | DIETARY INTAKE TERMINAL 7 DAYS | PITUITARY CYTOLOGY (PERCENTAGES) ACIDOPHILS STORAGE | ACIDOPHILS SECRETORY | HYPERSECRETORY CELLSS | BASOPHILS STORAGE | BASOPHILS SECRETORY | CHROMOPHOBES | ADRENALS | THYROID | GONADS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14. | 47-F | ULCERATIVE COLITIS 9 YRS.** | 43 K 162 cm | 14 K | POOR | 35.9 | 27.5 | 19.4 | 10.3 | 0.7 | 6.2 | 22.0 gm SLIGHT LIPOID DEPLET'N | 13.0 gm MOD. INVOLUTION | ABSENT |
| 15. | 27-F | ULCERATIVE COLITIS 9 YRS.** | 51 K 162 cm | 14 K | POOR | 42.3 | 26.7 | 10.4 | 12.1 | 3.6 | 4.9 | 18.0 gm MODERATE LIPOID DEPLET'N | 9.5 gm SLIGHT INVOLUTION | NORMAL |
| 16. | 63-F | GASTROINTEST. RESECT. 1 YR. * | 43 K 153 cm | 20 K | POOR | 39.9 | 30.0 | 17.4 | 7.1 | 0.6 | 5.0 | 13.0 gm MODERATE LIPOID DEPLET'N | 18.5 gm MOD. INVOLUTION | ATROPHY ▷ |
| 17. | 39-F | CHRONIC GLOMERULONEPHRITIS 4 MOS. * | 43 K 156 cm | 14 K | POOR | 50.6 | 14.9 | 5.8 | 18.5 | 1.6 | 8.6 | 10.0 gm NORMAL | 9.0 gm SEVERE ATROPHY | ATROPHY |
| 18. | 40-M | CHRONIC GLOMERULONEPHRITIS 2 YRS. * | 72 K 182 cm | 15 K | POOR | 51.9 | 18.0 | 8.6 | 15.9 | 1.6 | 4.0 | 18.0 gm NORMAL | 21.0 gm MODERATE ATROPHY | SPERMATOGENIC ARREST |
| 19. | 22-M | SYSTEMIC GRANULOMATOUS DISEASE 6 MOS. | 40 K | 20 K | NIL | 18.4 | 45.4 | 17.5 | 12.3 | 1.9 | 4.5 | MARKED LIPOID DEPLET'N | MARKED INVOLUTION | SPERMATOGENIC ARREST |
| | | NORMAL RANGE | | | | 44.3 TO 60.0 | 1.2 TO 2.8 | 0.0 TO 0.3 | 8.6 TO 16.8 | 6.5 TO 10.1 | 21.4 TO 34.7 | | | |

* = ADRENAL STEROID ** = ACTH TREATMENT ▷ = POSTMENOPAUSAL

## DISCUSSION

The adenohypophyses of thirteen patients dying of neoplastic disease showed a markedly altered cytological pattern compared with the normal adult gland (Table 1). There were strikingly increased percentages of cells characterized histologically by large, active nuclei and cytoplasm containing delicate orangophilic granules ("secretory acidophils") and still more active appearing cells with densely hyperchromatic nuclei and little visible cytoplasmic granulation ("hypersecretory cells"). The increased proportions of these cellular elements was at the expense of heavily granulated ("storage") acidophils and small inactive chromophobes.

The early findings of Severinghaus[14] as well as the more recent studies by Pearse[8] and Russfield[12] indicate that small densely granulated adenohypophyseal cells are primarily storage cells and that secretory activity can be attributed to cells which appear cytologically active, display prominent nuclei, and contain delicate cytoplasmic granulation. The bulk of morphological observations in patients with altered pituitary functional states supports this concept.

The morphological data in our patients point to a markedly increased secretory activity of acidophilic cells. Since these cells are generally accepted as the site of growth hormone secretion, the observations may reflect an enhanced secretion of this substance.

The possibility that growth hormone secretion is increased in the presence of severe dietary restriction has been recently discussed.[4, 13] Starvation reduces protein breakdown and nitrogen loss. Carbohydrate utilization is also minimized, with consequent depression of glucose tolerance. Fat, as a result, becomes the major metabolic substrate. The administration of primate growth hormone to human beings reproduces these changes, since it also causes increased protein synthesis and a positive nitrogen balance.[1, 5, 9] Blood glucose levels tend to rise (at least in diabetics) and glucose tolerance is reduced in normal subjects.[9] The hormone also promotes the mobilization of depot fat in the form of free fatty acids[9, 10] and may produce ketosis, changes which shift the metabolic pattern to one in which fat is the major source of energy. The close parallelism between the metabolic effects of starvation and the administration of growth hormone suggests that part of the adjustment to starvation is the result of secretion of this hormone.[13]

The morphological finding of increased secretory activity of the acidophil cells in our patients is consistent with an increased growth hormone secretion in response to restricted caloric and protein intake. Figure 1 shows that a close correlation exists between the degree of increased acidophil secretory activity and dietary intake during the seven days before death. Those patients who ate well during this period showed relatively slight cytological changes, whereas those who had little or no caloric or protein intake were found to have the highest percentages of secretory acidophils and hypersecretory cells. It is apparent from Table 1 that the terminal dietary intake was a much more significant factor in relation to the cytological changes than the history of weight loss or the terminal body weight.

The findings in the group of patients dying of non-neoplastic chronic disease associated with malnutrition (Table 2) support our interpretation. The increased percentages of secretory acidophils and hypersecretory cells in these patients are entirely comparable to those of the neoplastic group.

The importance of dietary intake during the relatively brief period preceding death is further emphasized by the findings of similar cytological changes in our study of patients dying in uremia of acute renal tubular necrosis.[4] These patients, previously in good health, died seven to twenty-one days after the onset of renal failure. They were maintained during their illness on a limited caloric intake derived solely from carbohydrate solutions.

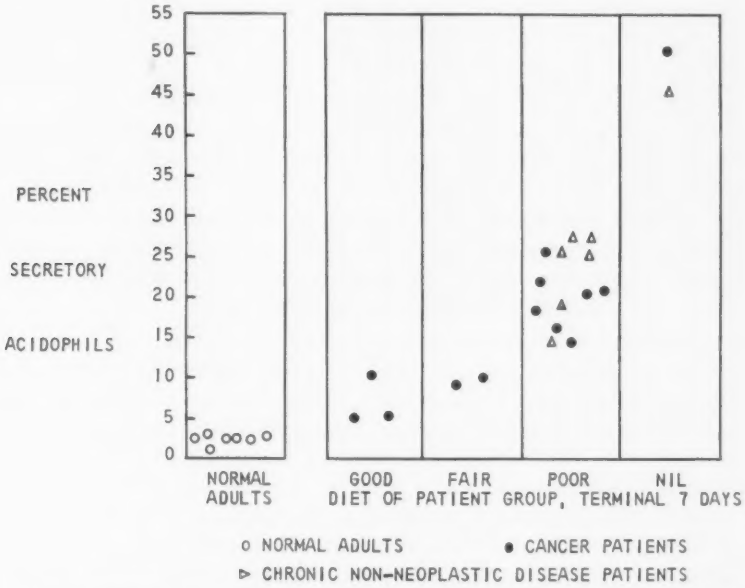


FIG. 1. Relation of acidophil secretory activity to diet during last seven days of life. Diets characterized on basis of estimated average daily intake: *good;* over 1,000 calories and more than 40 gm. protein, *fair;* 500-1,000 calories including 15-40 gm. protein, *poor;* less than 500 calories and less than 15 gm. protein, *nil;* few, if any calories and no protein.

It has been suggested that enhanced growth hormone secretion may play a role in the genesis of certain neoplasms.[15] The occurrence of increased acidophil secretory activity in non-neoplastic acute and chronic disorders associated with malnutrition as well as in patients dying of malignant tumors does not lend support to this concept. Although there is some evidence of a carcinogenic action of growth hormone in experimental animals,[11] it is of interest that a statistical study of patients with acromegaly (in whom pro-

longed excessive growth hormone secretion can be assumed) failed to reveal an increased incidence of malignant neoplasms.[8]

Consistent alterations were not noted in the basophilic cells, although there was morphological evidence of decreased secretion in eight of the thirteen patients (Table 1). In our previous study, we postulated that suppressed basophil secretion in patients dying of acute renal failure could result from either malnutrition or renal excretory failure per se. The importance of the latter factor is emphasized by the present findings. Crooke's change was present in the storage basophils of those patients receiving adrenal steroids, but no changes could be regularly correlated with the administration of other therapeutic hormone preparations.

The findings in the thyroid glands and gonads generally indicated decreased activity. The thyroid glands of most patients showed involution and in some, fibrosis and atrophy. The gonads showed decreased activity in all instances, although five of the female patients were post-menopausal. Some adrenal cortices appeared normal in structure and lipoid content, but most of the glands displayed varying degrees of lipoid depletion, an alteration which may reflect decreased functional activity.

It is concluded that the cytological changes observed in the adenohypophyses of our patients dying of neoplastic disease reflect altered pituitary function due primarily to malnutrition during the terminal period of life, and may represent the morphological expression of enhanced growth hormone secretion. The findings do not support a role of growth hormone in the genesis of the neoplasms of these patients since comparable changes were found in the hypophyses of patients dying of non-neoplastic disease associated with malnutrition. It is apparent that nutritional status, particularly during the period preceding death, can profoundly alter normal adenohypophyseal cytology and must be taken into account in the evaluation of all human pituitary glands.

SUMMARY

The adenohypophyses of thirteen patients dying of neoplastic disease showed morphological evidence of markedly increased secretory activity of acidophilic cells. This finding, which may reflect enhanced growth hormone secretion, was interpreted as a response to malnutrition, and could be correlated with the severity of caloric and protein deprivation during the last seven days of life. The importance of nutritional status in the evaluation of human pituitary cytology is emphasized.

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J. S. MEYER*
H. IMAI**
H. W. SCOTT†

*Department of Pathology, Washington University School of Medicine, St. Louis, Missouri*

# HEPATIC LIPID IN GOLDTHIOGLUCOSE-OBESE MICE‡

For several years we in this laboratory have been interested in the effects of obesity on the liver. Accumulations of fat have been observed in the livers of rats with insulin-induced polyphagia[21] and also in rats with hypothalamic polyphagia and obesity.[23] In both cases, the fat was present periportally rather than centrolobularly.

Elevation of hepatic lipid content has been described by several observers in mice which became obese after receiving goldthioglucose. Brecher and Waxler,[3] in reporting the initial observations of goldthioglucose-induced obesity, noted that abnormal amounts of stainable lipid were present centrolobularly in the livers of the obese mice. Subsequently the accumulation of fat in livers of obese mice has been confirmed histologically and biochemically in several laboratories,[5, 9, 10] but except for the initial report by Brecher and Waxler,[3] we have found no description of the lobular distribution, nor published photomicrographs. The study reported here was undertaken to investigate further the effects of goldthioglucose-induced obesity on hepatic lipid and to determine whether the fatty change could be attributed to a toxic effect of the goldthioglucose rather than to polyphagia and obesity.

## MATERIALS AND METHODS

Two experiments were performed using male, CFW, albino mice. They were kept in individual cages at 24-26° C. and were given a commercial stock diet‡ and water

* Formerly Assistant Resident. Presently Instructor of Surgical Pathology, Washington University School of Medicine.

** Formerly Assistant Resident. Present address: Department of Pathology, Union University, Albany Medical College, Albany 8, New York.

† Formerly Assistant Resident. Present address: Department of Radiology, University of Pennsylvania Hospital, Philadelphia, Pennsylvania.

This study was supported by grants CRT-5017 and C-2548 from the United States Public Health Service.

‡ Rockland Mouse Diet, A. E. Staley Mfg. Co., Decatur, Ill. The following data are given by the manufacturer. Composition: soybean oil meal, fish meal, dried whole milk, irradiated brewers type yeast, ground oats, wheat bran, wheat standard middlings, ground yellow corn, ground barley, feeding oat meal, ground whole wheat, dehydrated alfalfa meal, vitamin A feeding oil, ½ per cent steamed bone meal, 1 per cent calcium

*Received for publication 9 September 1960.*

ad libitum. Goldthioglucose was injected intraperitoneally in a concentration of 100 mg. per ml. of sesame oil.* At the termination of each experiment, the mice were weighed and were killed by decapitation. Blocks of liver were fixed in formalin containing 100 gm. each of calcium chloride and cobaltous nitrate per liter. Sections were cut with a freezing microtome and were stained for fat with oil red O.[28] Blocks of livers of selected animals from Experiment II were fixed for one hour in Dalton's chrome-osmic acid fixative, washed, dehydrated in alcohol, and embedded in a solution of 1 part butyl methacrylate in 7 parts of methyl methacrylate with benzoyl peroxide added as the polymerizing agent. Polymerization was carried out at 60° C. Small central veins were located by phase microscopy, and each block was trimmed to leave only the tissue including and immediately around a central vein. After thin sections were cut using a Porter-Blum microtome, the centrolobular localization was confirmed again by phase microscopy. The thin sections were photographed with a RCA Model EMU-2B electron microscope. Blocks from the same livers were also fixed in Carnoy's solution and paraffin sections were stained for glycogen by the PAS technique using duplicate sections digested by diastase as controls.

Experiment I utilized 200 mice. One hundred sixty received one or more injections of goldthioglucose. For the 35 injected mice that survived until the termination of the experiment, the initial doses ranged from 0.43 to 1.1 mg. of goldthioglucose per gm. body weight. Animals that survived for one month without abnormal weight-gain were given a second dose of goldthioglucose, 20 mg., intraperitoneally. Five mice received a third dose, 20 mg., after one more month. Thirty-eight mice were not given goldthioglucose and served as controls. Nineteen of them survived to the termination of the experiment. The mice initially were divided into two dietary groups, with each group made up of one-half of the injected and one-half of the uninjected mice. One group received a diet marginal in lipotropic adequacy for albino rats but otherwise sufficient in all essential dietary constituents.[23] The second group received a similar diet supplemented with choline chloride to a level of 0.5 per cent by weight. During the last four months, all mice received the commercial diet. At autopsy the mice showed no apparent differences attributable to the initial differences in diets.

Experiment II, in which 84 mice were used, was performed to extend the observations of Experiment I. Thirty-seven mice received a single injection of goldthioglucose when approximately three months old and weighing 21-30 gm. Ten received 1 mg. per gm., 10 received 1.5 mg. per gm. and 17 received 2.0 mg. per gm. Fourteen mice of similar

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carbonate, and 1 per cent salt. Average analysis (per cent): protein 21.93, fat 3.15, fiber 4.86, carbohydrates 56.23, nitrogen-free extract 52.29, and ash 7.78. Mineral composition (per cent): silicon dioxide 0.59, chlorine 0.70, sodium 0.41, potassium 0.72, calcium 1.68, phosphorus 0.80, sulphur 0.24, magnesium 0.33, manganese 0.0084, iron 0.019, zinc 0.002, copper 0.0012, boron 0.00137, cobalt 0.000035, iodine 0.0012, and fluorine 0.0019. Vitamin content per 100 gm.: vitamin A 610-680 U.S.P. units, thiamine 228-465 μg, riboflavin 436-650 μg., pyridoxine 209-275 mcg., pantothenic acid 1000-1594 μg., choline equivalent 480-492 mg., inositol (free) 61 mg., vitamin D 148 U.S.P. units, vitamin E 1.2 international units, PABA 78 μg., niacin 6.60 mg., vitamin C 3.6 mg., carotene 0.42-0.45 mg., and choline 132 mg. Representative amino acid content (per cent): lysine 1.15, methionine 0.40, cystine 0.29, tryptophane 0.27, arginine 1.39, histidine 0.53, isoleucine 1.20, leucine 1.74, phenylaline 1.03, threonine 0.87, valine 1.14, and tyrosine 0.72.

* The goldthioglucose was generously supplied by Preston H. Perlman, Ph.D., Director, Biochemical Research, Schering Corporation, Bloomfield, New Jersey.

age and weight were uninjected controls. Surviving animals were autopsied after nine weeks.

To investigate possible toxic effects of goldthioglucose or its sesame oil vehicle on the liver, two more groups of mice were used. They were similar to the others with respect to age and weight. Each member of a group of 14 received ⅓ mg. goldthioglucose per gm. body weight intraperitoneally weekly until the experiment was terminated after nine weeks. To explore the acute effects of goldthioglucose, each member of a group of 19 mice was killed or allowed to die spontaneously within four days after intraperitoneal injection of goldthioglucose. Ten received 1 mg. per gm. of body weight, and 9 received 2 mg. per gm.

The heaviest uninjected mouse in Experiment I weighed 42 gm., and all mice that weighed 45 gm. or more were arbitrarily designated obese. The short duration of Experiment II did not permit maximum differences in body weight between obese and nonobese animals to develop; consequently obesity was defined in terms of weight-gain (10 gm. or more) rather than in terms of final body weight. No uninjected control gained more than 7 gm.

Concentrations of stainable hepatic lipid were estimated for periportal and centrolobular locations by a method previously reported from this laboratory.[22] The grading system was as follows: Grade 0—no fat recognizable at 100 x magnification. Grade 1—fat present, but in no more than 50 per cent of cells. Grade 2—fat present in more than 50 per cent but in less than 85 per cent of cells. Grade 3—fat present in more than 85 per cent of cells, but no more than 15 per cent of cells heavily laden with fat (*i.e.*, 50 per cent or more of cytoplasm composed of fat). Grade 4—more than 15 per cent but not more than 50 per cent of cells heavily laden with fat. Grade 5—intermediate between Grade 4 and Grade 6. Grade 6: more than 85 per cent of parenchymal cytoplasm in area under consideration filled with fat. Actual counts of cells were not made, but this method of ranking provided a reliable basis for semiquantitative estimation of fat-content. Livers were examined by one of us (JSM) without knowledge of the identities of the animals from which they came.

The numerical data were analyzed by means of the *t* test adapted to the analysis of small groups.[23]

## RESULTS

In both experiments, obesity as determined by body weight was associated with centrolobular deposition of stainable lipid (Fig. 1 and Tables 1 and 2). Differences in the amounts of centrolobular lipid between goldthioglucose-obese mice and mice that received goldthioglucose but did not become obese are highly significant at the $P<0.001$ level of confidence in both Experiments I and II. Differences between the obese mice and the uninjected controls are also highly significant ($P<0.001$). The centrolobular accumulation of fat associated with obesity was generally proportional to the degree of obesity with a few exceptions. Significant periportal accumulation of stainable lipid did not occur in any obese mouse. The largest amount of periportal lipid occurring in an obese mouse was Grade 2, and in that mouse

TABLE 1. EXPERIMENT I—SUMMARY OF DATA

| Group | Number of mice | Mean initial body weight gm. | Mean final body weight, gm. | Mean weight of liver, gm. | Mean stainable hepatic lipid* | |
|---|---|---|---|---|---|---|
| | | | | | Centrolobular | Periportal |
| Obese, goldthioglucose-injected | 12 | 23.0 | 50.6 | 2.24 | 2.7 | 0.0 |
| Non-obese, goldthioglucose-injected | 23 | 21.6 | 32.6 | 1.63 | 1.0 | 0.2 |
| Non-obese, uninjected** | 20 | ... | 32.7 | 1.53 | 0.5 | 0.0 |

TABLE 2. EXPERIMENT II—SUMMARY OF DATA

| Group | Number of mice | Mean initial body weight gm. | Mean final body weight, gm. | Mean weight of liver, gm. | Mean stainable hepatic lipid* | |
|---|---|---|---|---|---|---|
| | | | | | Centrolobular | Periportal |
| Obese, goldthioglucose, single injection | 9 | 26.6 | 41.9 | 2.3 | 3.7 | 0.8 |
| Non-obese, goldthioglucose, single injection | 16 | 25.6 | 31.4 | 1.7 | 2.2 | 0.0 |
| Non-obese, goldthioglucose, multiple injections | 13 | 25.8 | 31.2 | 1.8 | 2.0 | 0.2 |
| Non-obese, uninjected | 13 | 27.1 | 31.4 | 1.8 | 0.8 | 0.1 |
| Non-obese, goldthioglucose, autopsied acutely | 19 | 25.7 | 23.6 | 1.4 | 0.6 | 1.3 |

* Estimated microscopically as described in text.
** Records of initial body weights were lost.

the centrolobular grade was 4. In none of the obese mice did the degree of periportal fatty change equal that in the center of the lobule.

Obesity was limited to mice that received injections of goldthioglucose larger than 0.6 mg. per gm. of body weight. None of the mice of Experiment II that were given nine weekly injections of 0.33 mg. of goldthioglucose per gm. of body weight became obese.

The centrolobular lipid in the livers of obese mice occurred as small droplets within the cytoplasm of parenchymal cells. The droplets usually remained small and failed to coalesce even in very fatty cells (Figs. 5 and 6). By light microscopy, the fat had a finely bubbly appearance instead of the homogeneous appearance characteristic of large globules. Fatty cysts were extremely rare. Fat-laden hepatic parenchymal cells of obese mice maintained their polygonal outlines, and their nuclei were usually centrally located. The fat-laden cells were enlarged, but not distorted by their content of lipid.

By electron microscopy thin septa could be seen separating the small globules of fat (Fig. 9). These septa consisted of cytoplasm in which ergastoplasmic membranes or other organelles could not be identified. The fat was not consistently related to mitochondria. Most of it had been lost during the preparation of the tissue, but fat-containing vacuoles usually retained small amounts of osmophilic material.

Mitochondria of the centrolobular parenchymal cells of obese, fatty livers were in no way different from those of nonobese, nonfatty livers of injected or noninjected animals (Figs. 11 and 12). Not only were changes in mitochondria not demonstrable among different animals, but also when mitochondria of fatty and nonfatty cells in the same animal were compared no differences were found.

The amount and distribution of stainable lipid in livers of the mice that received multiple doses of goldthioglucose to a total of 3 mg. per gm. of body weight and in the livers of nonobese mice that received single injections were similar. Small amounts of centrolobular lipid were observed in most livers, and the fat was contained in small vacuoles in parenchymal cytoplasm (Figs. 2 and 3).

In Experiment II, the amount of centrolobular lipid in livers of nonobese, injected mice and multiple-injected mice was significantly in excess of the amount in livers of the noninjected mice ($P<0.01$ for both comparisons). A few of the noninjected mice showed very small amounts of hepatic lipid, but most were completely devoid of it (Fig. 4). In Experiment I, livers of 12 of the nonobese, injected mice contained no stainable lipid, and in Experiment II, three of the nonobese, injected mice, including two that received

multiple injections, showed no stainable hepatic lipid. The difference in amount of centrolobular lipid between the two nonobese groups of Experiment I is not significant.

In the experiment designed to test the acute effects of goldthioglucose, the mice that were given 1.0 mg. per gm. of body weight all survived until they were killed two or four days after injection. No stainable lipid was observed in their livers. Nine of the mice had lost weight during the period after injection, and one showed no change in weight.

Five of the mice receiving 2.0 mg. of goldthioglucose per gm. body weight died within 24 hours after injection, and 2 more were killed at that time because they were moribund. The livers of these animals contained variable amounts of stainable lipid, but it was always located periportally rather than centrolobularly. The 2 remaining mice were killed four days after injection, and neither showed any stainable hepatic lipid. Thus of the 19 mice observed for acute effects of large doses of goldthioglucose on the liver, only those that died or were killed when moribund showed any fatty change, and the lipid that did occur was chiefly periportal.

Yellow pigment was present consistently in Kupffer cells of the mice that had received goldthioglucose (Fig. 8). It was not seen in uninjected animals. In general, the amount of pigment was proportional to the dose of goldthioglucose. The greatest quantities were found in livers of the mice that had received 3 mg. per gm. body weight. The pigment gave a positive reaction when stained by the stannous chloride method for gold[12] (Fig. 7). It did not contain stainable iron. It was insoluble in alcohol and xylol, PAS–negative, and oil red O failed to stain it in sections cut from tissue embedded in paraffin. These observations indicate that the pigment contains gold, and that it is free of ceroid and hemosiderin. Trace amounts of ceroid were found in Küpffer cells in all experimental groups and in controls without significant variation among groups.

The gold-containing pigment was distributed evenly throughout the hepatic lobule in contrast to the pericentral localization of fat. In electron micrographs it was extremely dense, and some deposits had crystalloid patterns (Fig. 10).

The PAS-stained sections showed no significant variations of hepatic glycogen content or its lobular distribution among the different groups in Experiment II. Most livers contained abundant glycogen. Livers of the mice killed acutely were not stained for glycogen.

In both Experiment I and Experiment II, the livers of obese animals were heavier than the livers of nonobese animals. The differences are significant at the $P<0.001$ level in Experiment I.

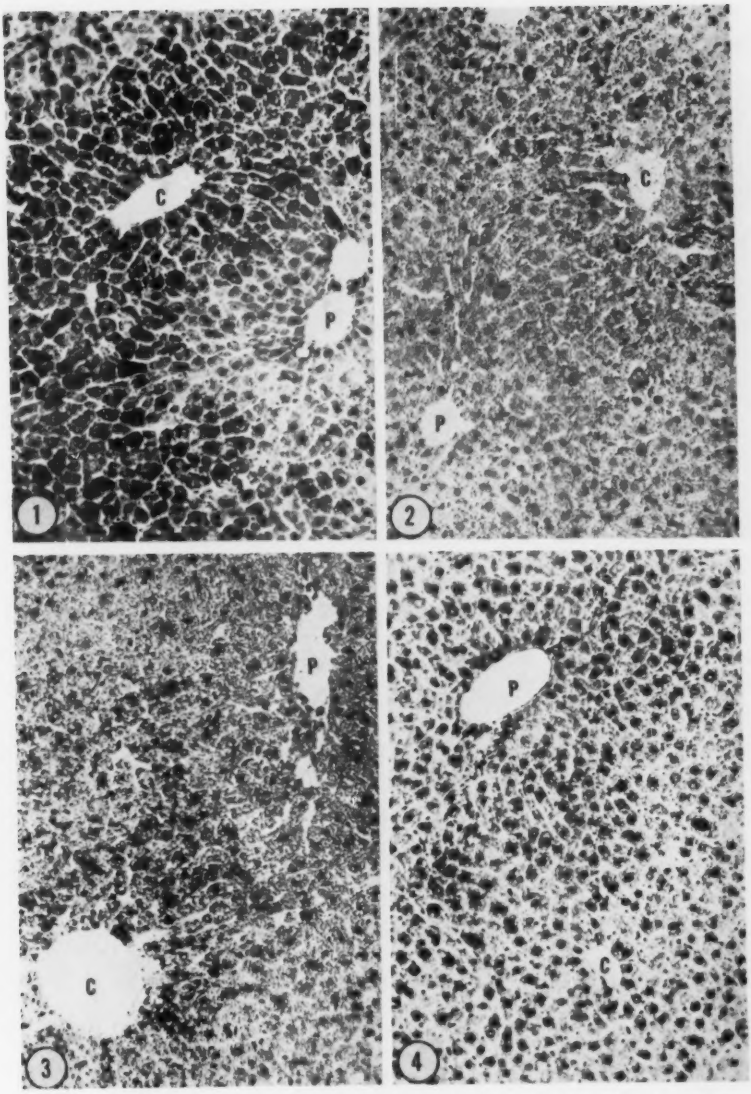
In Experiment II, the difference between the mean weights of the livers of the obese and the once-injected, nonobese mice are significant at the $P<0.001$ level, and the differences between the obese and the uninjected, nonobese are significant at the $P<0.01$ level. The differences between the hepatic weights of the obese mice and the multiple injected, nonobese mice are also significant at the $P<0.01$ level. In neither experiment did the weights of the livers of the nonobese groups differ significantly.

## DISCUSSION

The observation originally made by Brecher and Waxler that abnormal amounts of lipid accumulate centrolobularly in the livers of goldthioglucose-obese mice is confirmed. The data suggest that obesity, rather than a toxic effect of goldthioglucose or its sesame oil vehicle, is responsible for most of the fatty change.

Although increases in centrolobular stainable lipid occurred in some nonobese, injected mice relative to the noninjected controls, the degree of fatty change was not proportional to the dose of goldthioglucose given either as single or multiple injections. Mice killed or dying acutely following injection of goldthioglucose did not show increased centrolobular stainable lipid. These observations suggest that the minimal centrolobular fatty changes in livers of nonobese, injected mice may not have been due to an hepatotoxic effect of the goldthioglucose. It is possible that the arbitrary criteria based on body weight, or weight gain, failed to detect all of the obese mice with the result that a few animals in the nonobese, injected groups might actually have been slightly polyphagic and obese. Further evidence against a postulate that the hepatic fatty change might represent a toxic effect of goldthioglucose is the lack of any consistent juxtaposition of the sites of deposition of the stainable gold and the lipid. Gold was found only within Kupffer cells and was distributed randomly throughout the hepatic lobule. Lipid occurred in parenchymal cells and was concentrated centrolobularly. The data do not, however, definitely exclude an hepatotoxic effect of the goldthioglucose as a cause of a portion of the hepatic fatty change. If an idiosyncratic type of toxicity were operating, the effects on the liver would not necessarily be proportional to the dose of goldthioglucose.

With the exception of lobular distribution, the fatty change in the livers of goldthioglucose-obese mice resembled that in insulin-polyphagic rats and in rats made polyphagic and obese by hypothalamic electrocoagulation. In all cases lipid was deposited in the cytoplasm of parenchymal cells as small, discrete droplets. The droplets showed little tendency to coalesce, even in very fatty cells, and fatty cysts as seen in choline deficiency[4,7] were absent or




FIGS. 1-4. Representative livers of mice from Experiment II. In each of the figures, the structure nearest the center of the plate is a portal area (P) and the structure farthest from the center is a central vein (C). Lipid appears black. Frozen sections, oil red 0, hematoxylin, and light green (x140).

FIG. 1. Single injection of goldthioglucose, obese. Abundant centrolobular lipid.

FIG. 2. Single injection, not obese. Small droplets of lipid near central vein.

FIG. 3. Multiple injections. Small droplets of lipid near central vein.

FIG. 4. No injections. Virtually no stainable lipid.

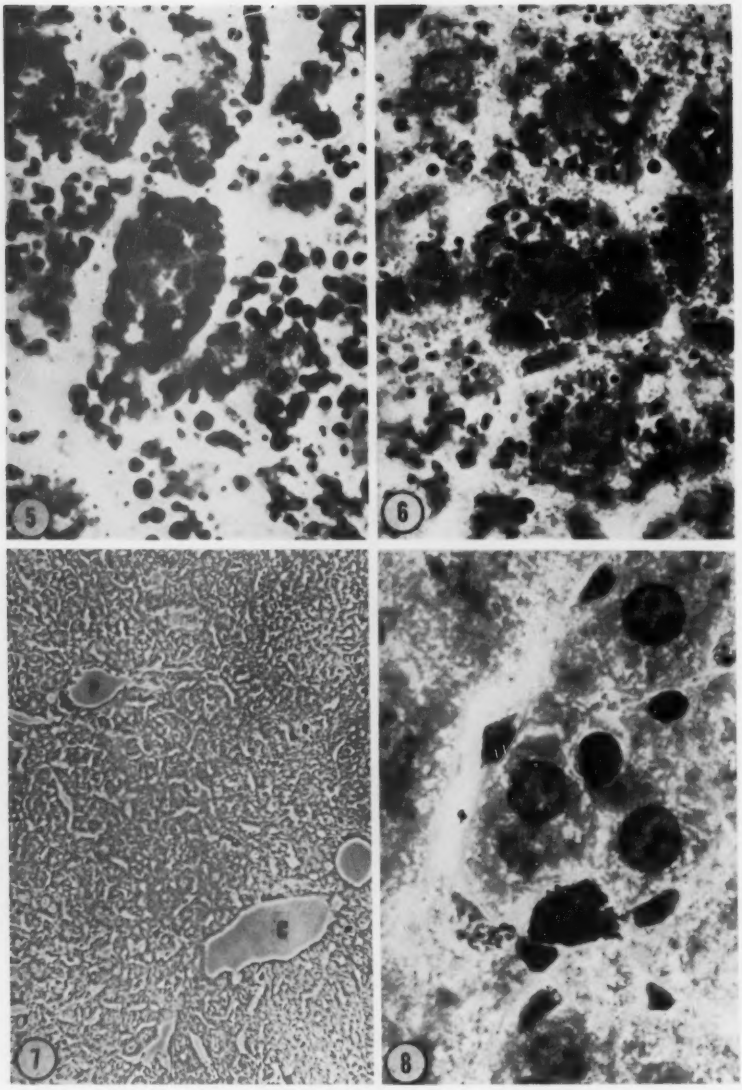



FIG. 5. Liver of obese mouse. Abundant lipid present as small droplets in parenchymal cells. Frozen section, oil red 0, hematoxylin, and light green (x1200).

FIG. 6. Liver of obese mouse. Intranuclear lipid in parenchymal cell. Frozen section, oil red 0, hematoxylin, and light green (x1200).

FIG. 7. Liver of mouse after multiple injections of goldthioglucose. Pigment in Kupffer cells stained black. Central vein (C) in lower right, portal area (P) in upper left. Phase contrast, $SnCl_2$ reaction (x140).

FIG. 8. Liver of mouse after multiple injections. Pigment in Kupffer cells. Oil red 0, hematoxylin, and light green (x1200).

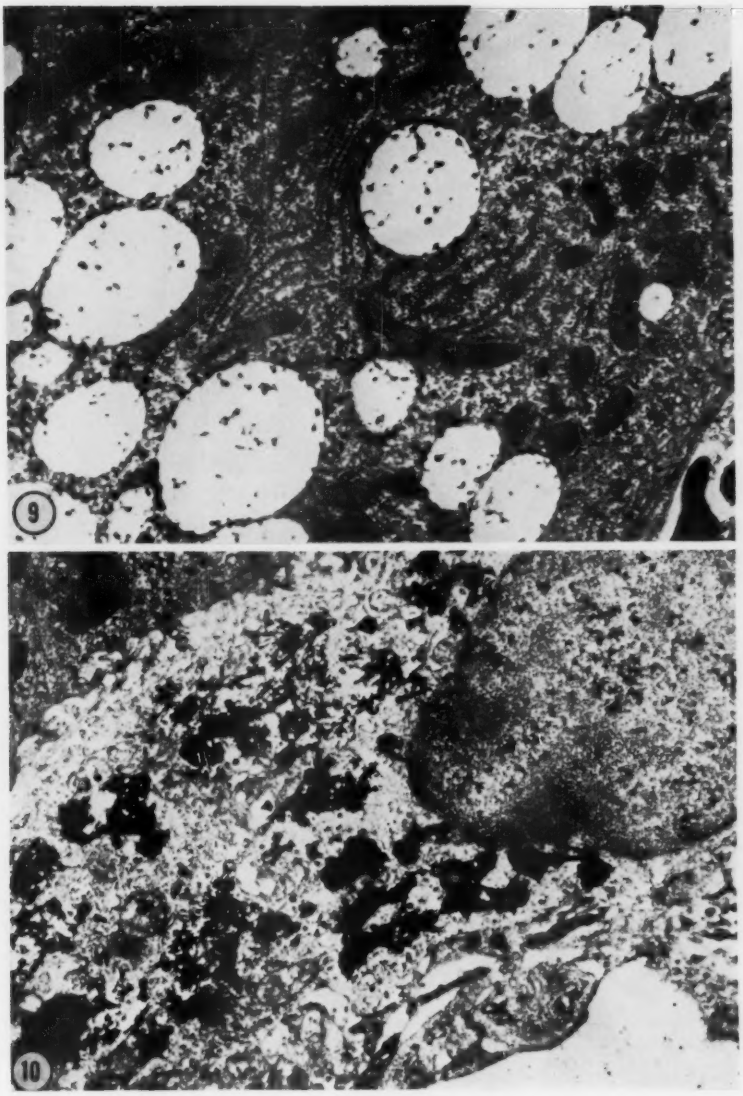



FIG. 9. Electron micrograph of parenchymal cell of liver of obese mouse of Experiment II. Narrow cytoplasmic septa separate adjacent vacuoles in which only small amounts of lipid remain after fixation and embedding. Mitochondria and ergastoplasm are normal (x10,000).

FIG. 10. Electron micrograph of Kupffer cell of mouse that received multiple injections of goldthioglucose. Gold-containing pigment has crystalloid pattern (x10,000).

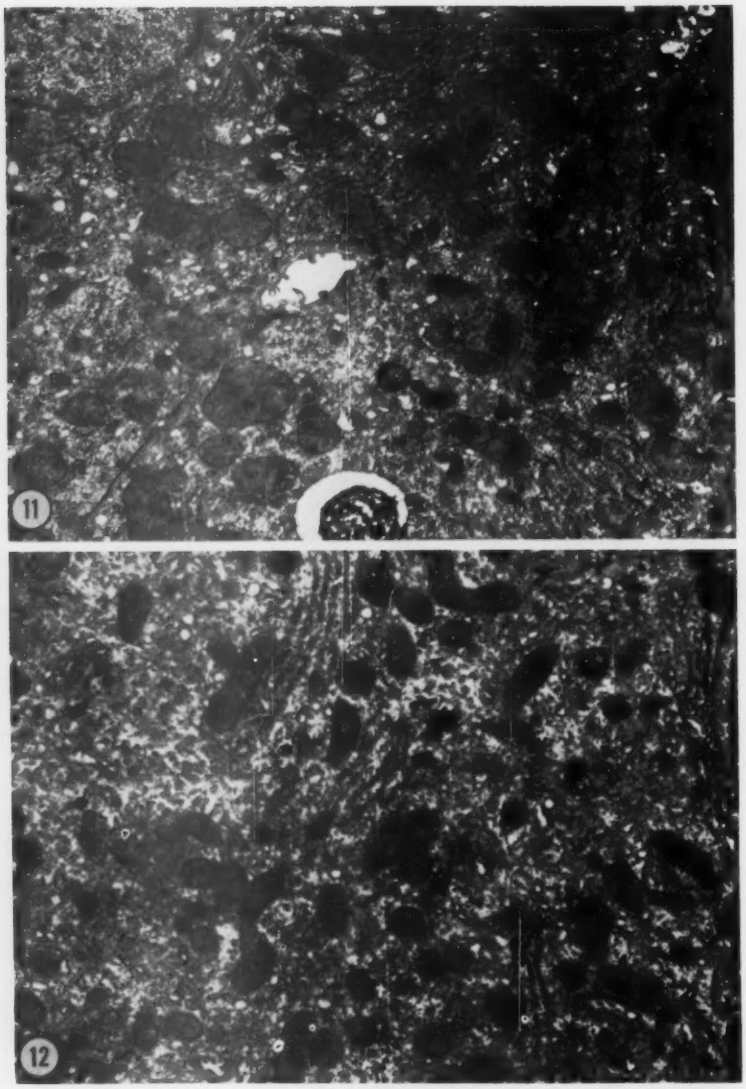



FIG. 11. Electron micrograph of parenchymal cell of liver of mouse that received multiple injections of goldthioglucose. A small lipid-containing vacuole is present (bottom). Mitochondria and ergastoplasm are normal (x10,000).

FIG. 12. Electron micrograph of parenchymal cell of liver of uninjected mouse. Mitochondria and ergastoplasm are similar to those in the preceding figures (x10,000).

rare. Fibrosis, necrosis, and disturbance of the lobular architectural pattern were absent. Intranuclear droplets of stainable lipid occurred in scattered cells in each condition.

The single feature that distinguished the fatty accumulation in livers of goldthioglucose-obese mice from that in insulin-treated[21] or hypothalamic-obese rats[23] was the lobular distribution of the excess lipid. In the rats, the fatty change was *always* preponderant in the periportal areas. An explanation for the difference is not apparent. One hypothesis that might be considered is that the hepatic lobules of rats and mice have different localizations of certain enzymatic or circulatory factors. A second possibility is that the difference in distribution of fat is a result of dietary factors. In choline-deficient mice, the type of dietary fat has been reported to influence the lobular localization of hepatic lipid.[25] The hypothalamic and insulin-treated rats received more saturated fat than the goldthioglucose-injected mice, but both received approximately the same dietary concentration of unsaturated fat. Neither diet was deficient in any known essential nutrient. With the exception that the rats received approximately 10 per cent more fat than the mice, distribution of calories among protein, fat, and carbohydrate, and the content of salts and fiber were similar. A third possibility is that the goldthioglucose, through a toxic effect, influenced the lobular site of deposition of lipid accumulating under the influence of polyphagia and obesity.

Choline deficiency under certain circumstances produces centrolobular fatty change in the livers of mice,[4, 20, 25] but we do not believe that the fat accumulation in the livers of goldthioglucose-obese mice is related to choline. No evidence of choline deficiency was present in controls, and the contents of protein, methionine, and choline were much higher than in diets used to produce choline deficiency. Hyperalimentation in rats did not raise the per cent dietary requirement of choline.[22]

Lesions in the ventromedial hypothalamic nuclei have been demonstrated consistently in goldthioglucose-obese mice. The lesions consist of edema, hemorrhage, and necrosis with loss of neurons and subsequent gliosis.[6, 11, 18] Since destruction of the ventromedial hypothalamic nuclei of mice[26] and of rats[8] by stereotaxic electrocoagulation also produces polyphagia and obesity, it seems well established that the effect of goldthioglucose on food intake and body weight is a result of total or partial destruction of appetite-inhibiting centers in the hypothalamus. Physiologically and biochemically, goldthioglucose-obese mice closely resemble hypothalamic-obese rats[6, 14, 15] in that their obesity is "regulatory" (increased caloric intake)[14, 18, 19, 24] rather than "metabolic" (increased basal rate of lipogenesis) as in certain other forms of obesity.[1, 2, 5, 14, 15, 17, 18, 19, 24] It is evident that at the present the difference in

hepatic reaction to polyphagia and obesity in goldthioglucose-obese mice and hypothalamic-obese rats cannot be attributed to differences in the metabolism of the two forms of obesity.

The dissimilarity in lobular position of lipid in livers of hypothalamic-obese rats and goldthioglucose-mice may be a result of a species difference in lobular localization of function, but the other possible explanations that have been discussed cannot be discarded at the present time. The evidence in favor of a species difference appears strong enough to necessitate more extensive investigation before observations of hepatic effects of obesity in one species can be generalized to other species.

## SUMMARY

Obesity following injection of goldthioglucose in mice is associated with centrolobular deposition of lipid in their livers and hepatomegaly. The lipid is deposited in parenchymal cells chiefly as small cytoplasmic droplets that bear no consistent relationship to mitochondria, ergastoplasm, or other organelles. Occasional nuclei also contain lipid. Neither the deposition of fat in the livers nor the hepatomegaly can be accounted for entirely on the basis of a direct (toxic) action of goldthioglucose; therefore, they appear attributable at least in part to the effects of polyphagia and obesity.

Hypothalamic polyphagia and obesity in rats are known to result in accumulation of hepatic fat in periportal location. The dissimilarity in the lobular site of lipid in the livers of goldthioglucose-obese mice and hypothalamic-obese rats may represent a species difference, or it may be a result of differences in the experimental treatment of the rats and the mice.

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LAWRENCE R. FREEDMAN*
PAUL B. BEESON**

*Department of Internal Medicine, Yale University School of Medicine*

# EXPERIMENTAL PYELONEPHRITIS

## VIII. THE EFFECT OF ACIDIFYING AGENTS ON SUSCEPTIBILITY TO INFECTION†

## INTRODUCTION

The experiments to be described were conducted with the aim of testing the suggestion of Beeson and Rowley,[1] that the peculiar susceptibility of the kidney to certain forms of bacterial infection may be dependent on local inactivation of complement by ammonia which is produced in renal tubular cells. This was based on the finding that kidney tissue has a considerably stronger anticomplementary action than other tissues because of inactivation of the fourth component of complement, a component known to be affected by weak concentrations of ammonia. Evidence was obtained that the kidney's anticomplementary action tended to parallel glutaminase activity. It appeared therefore that the hypothesis could be subjected to further test in living animals by determining whether stimulating ammonia formation by administration of acid loads increased susceptibility to bacterial infection in the kidney.

## MATERIALS AND METHODS

### *Animals*

Adult male Sprague-Dawley rats of comparable age and weight were used as the experimental animals.

### *Bacterial strains*

The strain of *E. coli* used has been maintained in this laboratory for five years and details of its handling have been described previously.[2] The standard infecting dose for animals was 0.5 ml. of a four-hour culture, containing 2-6x$10^8$ organisms per ml.

A hemolytic coagulase negative *Staphylococcus albus* of low virulence which had been obtained from a human throat was also used. Agar slant cultures were stored at 4° C. At weekly intervals a blood agar plate was inoculated and incubated; a single

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* Markle Scholar in the Medical Sciences and Assistant Professor of Medicine.
** Ensign Professor of Medicine.
† Supported by a grant from the United States Public Health Service—(E—1850)
A preliminary report of this work was presented at the Henry Ford Hospital, October 1959, at the Symposium, *The Biology of Pyelonephritis* (in press).
*Received for publication 3 October 1960.*

colony from this was used to inoculate 10 ml. of trypticase soy broth. After 12 hours of incubation at 37° C. the broth contained 2-4x$10^9$ bacteria per ml. Many experiments with this organism have shown that no observable infection, in the kidneys or elsewhere, follows intravenous injection of $10^5$ bacteria in normal animals. For the experiments involving animals with acid loads, the infecting dose was 0.5 ml. of the 12-hour culture, i.e. 1-2x$10^7$ organisms.

*Enumeration of bacteria in organs*

Under pentobarbital anesthesia, the abdominal and thoracic cavities were opened and the organs to be examined removed and placed in a Teflon tissue grinder. In the case of kidney or spleen, whole organs were used, whereas with the lung, approximately one gram of tissue was taken. Homogenates were made in 9 ml. of 0.9 per cent saline. Serial ten-fold dilutions were mixed with melted agar, placed in petri dishes, and incubated for 24-48 hours, after which, colony counts were made. Urine for culture was obtained by needle aspiration of the bladder at the time of sacrifice. One drop was streaked on a blood agar plate.

*Renal glutaminase*

Determinations for this enzyme were performed according to the method of Rector, Seldin, and Copenhaver[18] with the modification that KCN was not used.

*Criteria of infection*

Kidneys were considered to be infected when they contained 50,000 or more viable microbial units. They frequently showed gross evidence of abscess formation. Urine cultures were considered positive when 10 or more colonies were found on the streak plate. This is equivalent to at least $10^3$ to $10^4$ colonies per ml. of urine.

*Diet and drinking solutions*

The diet consisted of Purina Lab Chow pellets. The particular method of feeding will be described in connection with the individual experiments.

Solutions of various salts and amino acids for drinking water were made with tap water. Due to its relative insolubility, DL-methionine solution was prepared by dissolving 4.5 gm. in 100 ml. of hot water. This solution was stored (as was the DL-valine) in the refrigerator. The refrigeration of DL-methionine caused the precipitation of crystals which were then removed by filtration before giving the solution to the rats as drinking water. The precise concentration of DL-methionine in the drinking water probably did not exceed 3.38 gm./100 ml. water, since this is its solubility at 25° C.

*Urine pH*

An approximation of urine pH was obtained with nitrazene paper.

## EXPERIMENTAL

The simplest method of administering acid to rats is to provide ammonium chloride solution in place of drinking water. The ammonium is converted to urea in the liver, leaving the chloride ion to be excreted by the kidney. This is, therefore, in reality a convenient way of administering hydrochloric acid.

It causes increased renal glutaminase activity and augments ammonia production by renal tubular epithelial cells.[8]

The first experiment was conducted with 12 animals drinking 2 per cent $NH_4Cl$ and 6 control animals drinking an equal amount of water. These animals were fed the same amount of chow pellets. The average daily intake of $NH_4Cl$ by the test animals was 8.8 millimoles.

TABLE 1.

| | *Colonies E. coli* | | *Gross abscesses* | *Urine culture* | *Blood urea N mg.%* | *Serum chloride m.Eq/L* |
|---|---|---|---|---|---|---|
| | *Left kidney* | *Right kidney* | | | | |
| | 40,000 | 40,000 | 0 | 0 | | |
| | >1,000,000* | 1,000,000* | + | + | | |
| | >1,000,000* | 1,000,000* | ± | + | | |
| | 70,000* | 2,000 | 0 | 0 | | |
| | 30,000 | 200,000* | 0 | 0 | | |
| 2% | 500 | 1,000,000* | ± | + | | |
| $NH_4Cl$ | 80,000* | 200,000* | 0 | + | 41 | 116 |
| | 600,000* | 400,000* | + | 0 | 35 | 118 |
| | 200,000* | 80,000* | + | + | 37 | 118 |
| | 400,000* | 80,000* | + | + | 43 | 122 |
| | 40,000 | 30,000 | 0 | 0 | 41 | 120 |
| | 50,000* | 10,000 | 0 | + | 41 | 123 |
| | 3,000 | 700 | 0 | 0 | | |
| | 400 | 0 | 0 | 0 | | |
| Water | 0 | 0 | 0 | 0 | | |
| | 200 | 600 | 0 | 0 | 48 | 107 |
| | 4,000 | >1,000,000* | 0 | + | 43 | 109 |
| | 2,000 | 1,000 | 0 | 0 | 33 | 110 |

* Infection.

The standard inoculum of *E.coli* was injected intravenously into the 18 animals on the third day, and all were killed seven days later. Gross inspection and culture of the kidneys revealed numerous instances of pyelonephritis among the acid-fed rats whereas only 1 of 12 kidneys in the control group contained a significant number of bacteria. Details of these results are shown in Table 1.

The concentration of $NH_4Cl$ in the drinking water was found to be excessive, interfering with food and water consumption. During the period of the experiment weight losses were comparable; the test animals lost an

average of 30 per cent of body weight, as compared with 26 per cent in the pair-fed controls.

Because of the severe impairment of appetite and thirst which resulted from drinking 2 per cent $NH_4Cl$ solution, the concentration of this salt was

TABLE 2.

| | *Colonies E. coli* | | *Gross* | *Urine* | *Blood urea N* | *Serum chloride* |
|---|---|---|---|---|---|---|
| | *Left kidney* | *Right kidney* | *abscesses* | *culture* | *mg.%* | *m.Eq/L* |
| | 0 | 0 | ± | | 23 | 116 |
| | 1,000,000* | >1,000,000* | + | | 27 | 113 |
| | 10,000 | 0 | 0 | | 21 | 117 |
| | 80 | 0 | 0 | | 23 | 114 |
| | 0 | 9,000 | ± | | 22 | 114 |
| 1.6% | 100 | 3,000 | 0 | | 30 | 110 |
| $NH_4Cl$ | 500 | 10 | 0 | + | 17 | 106 |
| | 100 | 40 | 0 | 0 | | |
| | 40,000 | 60,000* | 0 | 0 | 28 | 107 |
| | 400 | 400,000* | + | | 23 | 110 |
| | 7,000 | >1,000,000* | + | + | 22 | 116 |
| | 400,000* | 30,000 | 0 | + | 19 | 111 |
| | 300 | 50 | 0 | | 34 | 97 |
| | 90 | 0 | 0 | | 32 | 98 |
| | 30 | 0 | 0 | | 24 | 100 |
| 2.3% | 0 | 2,000 | 0 | | 29 | 101 |
| $NH_4C_2H_3O_2$ | 0 | 0 | 0 | | 34 | 102 |
| | 20 | 3,000 | 0 | | 24 | 99 |
| | 0 | 0 | 0 | 0 | 15 | 98 |
| | 0 | 10 | 0 | 0 | 23 | 98 |
| | 0 | 10 | 0 | 0 | 16 | 102 |
| | 40 | 10 | 0 | 0 | 20 | 102 |
| | 30 | 0 | 0 | 0 | 23 | 103 |

* Infection.

reduced to 1.6 per cent in subsequent experiments. This was better tolerated and permitted the test animals to more nearly maintain their initial weights for the duration of the experiments.

*Comparison of effects of ammonium chloride and ammonium acetate*

To rule out an effect of the ammonium ion, an experiment was carried out in which animals drinking 1.6 per cent ammonium chloride were compared

with animals drinking equimolar ammonium acetate. This would supply the same amounts of $NH_4^+$, but the acetate would not produce systemic acidosis. The animals were comparably fed in groups of 6. *E. coli* were injected intravenously five days after the experiment began and bacteriological examination was carried out nine days later. As shown in Table 2, there were 6 infected kidneys in the $NH_4Cl$ group and none in the ammonium acetate group. The average weight loss of the latter group was 9.3 per cent whereas the $NH_4Cl$ group average weight was the same at the end of the experiment as it was at the start. Of the 5 infected animals, one had gained weight and only one had lost more than 10 per cent of its original body weight.

TABLE 3.

| *% Loss of body weight* | *Number kidneys infected* |
|---|---|
| 10 | 0/30 |
| 15 | 0/12 |
| 20 | 0/18 |
| 35-41 (LD-50) | 1/10 |

The effect of $NH_4Cl$ in increasing the susceptibility of the kidney appeared to be dependent upon the production of systemic acidosis with consequent increased renal $NH_3$ production rather than upon the administration of $NH_4^+$ in the diet.

*Effect of inanition*

Since many rats drinking $NH_4Cl$ lost weight, it was necessary to determine whether inanition alone would effect the susceptibility of the kidney to infection with *E. coli*. A group of rats was therefore underfed and allowed drinking water freely. After four to ten days they were inoculated intravenously with *E. coli* and sacrificed four to seven days later. As can be seen in Table 3, only when an extreme degree of weight loss was achieved did one of the surviving animals develop infection.

*Induction of acidosis two days after inoculation of bacteria*

It has been demonstrated that *E. coli* persist in the rat kidney for several days after the blood culture has become negative.[8] The following experiment was designed to test whether bacteria persisting in the normal kidney at a time the blood culture would be expected to be negative could produce an infection during acid administration. Two days after the inoculation of 41 normal rats with *E. coli* intravenously, 11 were started on 1.6 per cent

$NH_4Cl$; the remainder received equimolar solutions of $NaH_2PO_4$, ammonium acetate, NaCl, or urea.* Four to eight days after the inoculation of organisms bacteriological studies were carried out. None of the 30 control animals had infection in the kidneys or urine, whereas of the 11 rats drinking $NH_4Cl$, 3 kidneys of 2 rats were heavily infected. This experiment demonstrates that pyelonephritis may occasionally be produced even when $NH_4Cl$ administration is not begun until after the inoculation of *E. coli*.

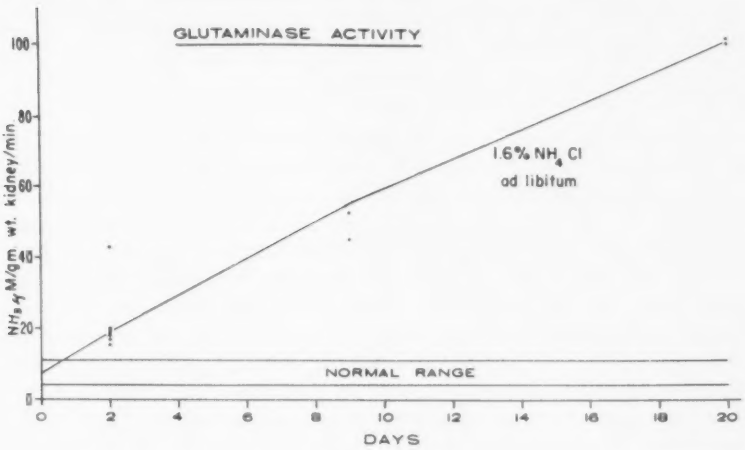


FIG. 1.

*Effect of varying duration of acidosis*

Administration of $NH_4Cl$ to rats results in a steady rise in glutaminase activity as illustrated in Figure 1. This progressive rise is associated with increasing oral intake of $NH_4Cl$. One would anticipate then that the incidence of infection would vary according to the duration of acidification. To test this point, 12 rats were given 1.6 per cent $NH_4Cl$ drinking water five days prior to infection. Another group of 12 rats, comparably fed with the first group, drank unlimited amounts of tap water until one day before receiving *E. coli* when they too had 1.6 per cent $NH_4Cl$ substituted for tap

* Although $NaH_2PO_4$ increases acid excretion, it does not cause a rise in renal glutaminase activity.[16] This salt would be an excellent control to distinguish the effects of acid secretion from those of $NH_3$ production. Unfortunately, it rapidly produces severe morphological damage to the kidney, thus complicating the interpretation of experiments designed to test susceptibility to infection.

water. Both groups drank $NH_4Cl$ for an additional three days at which time their kidneys were examined. Of the group drinking $NH_4Cl$ for eight days, 6 kidneys from 3 animals were heavily infected. In the group drinking $NH_4Cl$ for four days, no infections occurred.

It appears that the degree of susceptibility to infection induced by $NH_4Cl$ does have some relation to the duration of its administration. Had larger numbers of animals been used, one would have expected to find infections even in the animals receiving $NH_4Cl$ for four days since some susceptibility to infection was demonstrated in a previously described experiment in which $NH_4Cl$ was begun two days after the inoculation of *E. coli*.

*Reversal of effect of $NH_4Cl$ by alkalinizing agent*

If the effect of $NH_4Cl$ on renal susceptibility to infection is biochemical, and not dependent on structural alteration, this should be reversed by neutralizing the acidosis and abolishing the stimulus to renal production of ammonia. To test this, 54 rats were given 1.6 per cent $NH_4Cl$ as drinking water for seven days. The animals were then divided so that the half which had lost the most weight (18 per cent) were allowed to drink 2.5 per cent (equimolar) sodium bicarbonate, whereas the remainder (11 per cent weight loss) continued drinking $NH_4Cl$. After 24 hours, all animals were inoculated with *E. coli* and the same drinking solutions continued. The rats drinking $NaHCO_3$ were limited to 40 ml. per rat per day. Five to seven days later bacteriological examination of their tissues and body fluids were performed. The results are shown in Table 4. Among the rats which had been given $NaHCO_3$, 3 of 54 kidneys were infected. In the group which continued drinking $NH_4Cl$, 22 of 54 kidneys, representing 13 of 27 animals, were infected.

It is apparent from this experiment that the mechanism by which $NH_4Cl$, increases renal susceptibility to infection is largely nullified by the administration of an alkalinizing agent. The reduction in renal glutaminase activity which follows drinking 2.5 per cent $NaHCO_3$ for 24 hours is shown in Figure 2. It appears that renal susceptibility to infection under the conditions of this experiment varied with renal glutaminase activity.

*Effect of $NH_4Cl$ ingestion on the normal kidney*

To test for evidence of morphological abnormality in the kidneys of rats drinking $NH_4Cl$, urinalyses were performed on 12 animals seven and eight days after they had been drinking 1.6 per cent $NH_4Cl$ ad libitum. Evidence of renal injury in the form of abnormal proteinuria, hematuria, pyuria, or cylindruria was not observed. Bacteriological examination failed to reveal

TABLE 4.

| | Colonies E. coli | | Gross abscesses | Urine culture |
|---|---|---|---|---|
| | *Left kidney* | *Right kidney* | | |
| | 200 | 600 | | 0 |
| | 60,000* | 50,000* | | 0 |
| | 70,000* | >1,000,000* | + | + |
| | 700 | 200 | | 0 |
| | 0 | 300 | | 0 |
| | >1,000,000* | >1,000,000* | ± | + |
| | 2,000 | 70,000* | | 0 |
| | 400 | 40,000 | | 0 |
| | 1,000,000* | >1,000,000* | | + |
| | >1,000,000* | >1,000,000* | | + |
| $NH_4Cl$ | 420 | 740 | | |
| | 90 | 50 | | |
| | 0 | 0 | | |
| | 34,000 | 540 | | 0 |
| | 0 | 0 | | 0 |
| | 8,000 | 4,000 | | 0 |
| | 0 | 0 | | 0 |
| | 40 | 30 | | 0 |
| | 23,000 | >1,000,000* | | + |
| | 470 | 140 | | + |
| | >1,000,000* | >1,000,000* | | + |
| | >1,000,000* | >1,000,000* | | + |
| | 0 | 0 | | 0 |
| | 10,000 | >1,000,000* | + | + |
| | 600 | >1,000,000* | + | + |
| | 135,000* | >1,000,000* | + | + |
| | >1,000,000* | >1,000,000* | + | + |
| | 0 | 0 | | 0 |
| | 0 | 0 | | 0 |
| | 0 | 0 | | 0 |
| | 0 | 20 | | 0 |
| | 400 | 900 | | + |
| | 8,000 | >1,000,000* | | 0 |
| | 0 | 20 | | 0 |
| | 5,000 | 20,000 | | 0 |
| $NH_4Cl$ | 90 | 720 | | 0 |
| and | 470 | 5,000 | | 0 |
| $NaHCO_3$ | 70 | 170 | | 0 |
| | 6,000 | 7,000 | | 0 |
| | 610 | 940 | | 0 |
| | 24,000 | 3,000 | | 0 |
| | 68,000* | 13,000 | | 0 |
| | 8,0000 | 120 | | 0 |
| | 0 | 0 | | 0 |
| | 0 | 0 | | 0 |
| | 130 | 150 | | 0 |
| | 0 | 0 | | 0 |
| | 0 | 8,000 | | + |
| | 70 | 7,000 | | + |
| | 970 | 130,000* | | + |
| | 2,000 | 1,000 | | 0 |
| | 0 | 120 | | 0 |
| | 24,000 | 0 | | 0 |
| | 0 | 0 | | 0 |

* Infection.

the presence of bacteria in the kidneys or urine. Careful histological examination of all kidneys failed to reveal morphological evidence of renal injury. A group of 10 rats was given 1.6 per cent $NH_4Cl$ as drinking water for three weeks and then changed to 1.0 per cent $NH_4Cl$ which they drank for an additional two and one-half months prior to examination. The kidneys of these animals were sterile, and no abnormalities were seen grossly or microscopically. Insofar as these methods of examination can be relied

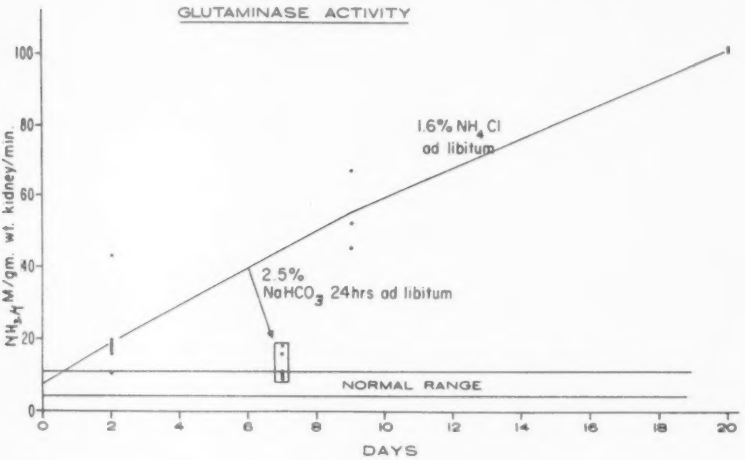


FIG. 2.

upon, there was no evidence that the effect of acid ingestion on susceptibility to infection is secondary to injury of kidney substance.

*Maintenance of effect of $NH_4Cl$ by methionine*

To test whether acidifying agents other than $NH_4Cl$ were effective in lowering renal resistance to infection, the effects of the amino acids DL-methionine and DL-valine were compared. Methionine produces an acid urine because of the excretion of sulfate.[9] Valine contains the same number of carbon and nitrogen atoms but does not contain sulfur and therefore would not be expected to acidify the urine. Forty-seven rats which had been drinking 1.6 per cent $NH_4Cl$ ad libitum for 11 to 20 days were divided into two groups of 24 and 23 animals. The $NH_4Cl$ drinking water was removed from all the animals and those which had lost weight (average 4 per cent) were given 3.5 per cent DL-valine to drink; those which had not lost weight

TABLE 5

| | Colonies E. coli | | Gross abscesses | Urine culture |
|---|---|---|---|---|
| | *Left kidney* | *Right kidney* | | |
| | 404,000* | >1,000,000* | + | + |
| | 460,000* | 100,000* | | 0 |
| | 0 | 0 | | 0 |
| | 1,000 | 2,500 | | 0 |
| | 14,000 | 62,000* | ± | 0 |
| | >1,000,000* | 1,000 | | + |
| | 130 | 0 | | 0 |
| | 0 | 0 | | 0 |
| | >1,000,000* | >1,000,000* | + | 0 |
| $NH_4Cl$ | 0 | 0 | | 0 |
| and | 0 | 0 | | 0 |
| Methionine | 50 | 190 | | 0 |
| | 148,000* | 208,000* | | 0 |
| | 1,000 | 70 | | 0 |
| | 36,000 | 10,000 | | 0 |
| | 0 | 0 | | 0 |
| | 74,000* | 62,000* | + | 0 |
| | 0 | 0 | | 0 |
| | 2,000 | 3,300 | ± | 0 |
| | 0 | 0 | | 0 |
| | 26,000 | >1,000,000* | + | + |
| | 1,600 | 0 | | 0 |
| | 1,600 | 20 | | 0 |
| | 6,000 | 175,000* | + | 0 |
| | 0 | 0 | | 0 |
| | 4,000 | 1,000 | | 0 |
| | 1,000 | 1,000 | | 0 |
| | 46,000 | 4,250 | | 0 |
| | 0 | 0 | | 0 |
| | 0 | 0 | | 0 |
| | 0 | 0 | | 0 |
| | 0 | 0 | | 0 |
| $NH_4Cl$ | 0 | 0 | | + |
| and | 720,000* | 1,300 | + | 0 |
| Valine | 0 | 0 | | 0 |
| | 0 | 40 | | + |
| | 3,000 | 13,000 | ± | 0 |
| | 1,000 | 10,000 | | 0 |
| | 5,000 | 186,000* | + | 0 |
| | 0 | 0 | | 0 |
| | 0 | 0 | | 0 |
| | 0 | 0 | | 0 |
| | 4,000 | 38,000 | | 0 |
| | 1,000 | 0 | | 0 |
| | 0 | 0 | | 0 |
| | 20 | 0 | | 0 |
| | 20 | 0 | | 0 |

* Infection.

were given DL-methionine. Twenty-four hours later all animals were inoculated intravenously with *E. coli*. The animals drinking DL-valine were allowed only as much fluid as had been taken by the animals drinking DL-methionine. After five to seven days when each group was 11 per cent below the average starting weight, the animals were examined bacteriologically (See Table 5). Infection was found in 14 kidneys of rats drinking DL-methionine and in 2 kidneys of animals drinking DL-valine.

This result indicates that the acidifying amino acid DL-methionine can maintain the biochemical susceptibility to infection resulting from $NH_4Cl$ ingestion. The amino acid DL-valine, on the other hand, which is not an acidifying agent, seemed to permit the animals to recover their resistance to infection.

*Methionine vs. valine*

Since methionine is being used in clinical practice to combat urinary tract infection by acidifying the urine, it seemed important to test whether DL-methionine alone could lower renal resistance to infection. Seventeen rats were given this amino acid in their drinking water for 15 days. At the same time 18 similarly fed rats were given equal volumes of drinking water containing 3.5 per cent DL-valine. All were then inoculated with *E. coli* intravenously and the pair-drinking and feeding continued for four to six days, at which time bacteriologic studies were carried out. As can be seen in Table 6, 3 rats drinking DL-methionine were infected whereas none of the animals drinking DL-valine was infected.

It would appear from the last two experiments that acidifying agents other than $NH_4Cl$ can increase renal susceptibility to infection.

*Staphylococcus albus*

In view of the similarities between coliform and staphylococcal pyelonephritis in animals,[4] the effect of acidification on renal staphylococcal infection was tested. As already stated, the strain chosen for this experiment does not produce pyelonephritis even when $10^8$ viable units are injected intravenously. Twenty-two rats were given 1.6 per cent $NH_4Cl$ as drinking water for four to eight days. An equal number of control animals was pair-fed to the experimental group and allowed similar quantities of tap water to drink. All animals were inoculated with $10^7$ staphylococci intravenously and examined bacteriologically four to seven days later. The average weight loss of the experimental and control groups at the time of inoculation was 3.9 per cent; at the time of sacrifice the weight losses were 4.6 per cent and 7.5 per cent respectively. Of the 22 rats drinking $NH_4Cl$,

TABLE 6

| | *Colonies* E. coli | | *Gross abscesses* | *Urine culture* | *Urine pH* |
|---|---|---|---|---|---|
| | *Left kidney* | *Right kidney* | | | |
| | 410 | 19,000 | | 0 | 6.0 |
| | 580 | 420 | | 0 | |
| | 650 | 320 | | 0 | 6.5 |
| | 4,000 | 24,000 | | 0 | 7.0 |
| | 16,000 | 5,000 | | 0 | 7.0 |
| | 20 | 20 | | 0 | 7.5 |
| Valine | 0 | 400 | | 0 | 7.5 |
| | 4,000 | 7,000 | | 0 | 6.5 |
| | 120 | 0 | | 0 | 7.0 |
| | 0 | 0 | | 0 | 7.0 |
| | 0 | 520 | | 0 | 6.0 |
| | 0 | 0 | | 0 | 6.0 |
| | 0 | 0 | | 0 | 7.0 |
| | 0 | 30 | | 0 | 6.5 |
| | 0 | 0 | | 0 | 6.5 |
| | 0 | 20 | | 0 | 6.5 |
| | 0 | 0 | | 0 | 6.5 |
| | 0 | 0 | | 0 | 7.0 |
| | 7,000 | 1,000 | | 0 | 6.0 |
| | 30 | 0 | | 0 | 5.5 |
| | 4,000 | 1,280 | | 0 | 6.0 |
| | 4,500 | 29,000 | | 0 | 6.0 |
| | 0 | 0 | | 0 | 5.0 |
| | 3,400 | 1,500 | | 0 | 5.0 |
| | 0 | 0 | | 0 | 5.0 |
| Methionine | 0 | 0 | | 0 | 5.0 |
| | 0 | 0 | | 0 | 5.0 |
| | 0 | 0 | | 0 | 5.5 |
| | 16,000 | 450,000* | + | + | 5.5 |
| | | 20,000 | | 0 | 5.5 |
| | 2,000 | 148,000* | + | + | 6.0 |
| | 0 | 0 | | 0 | 5.5 |
| | 0 | 0 | | 0 | 5.5 |
| | 30,000 | 154,000* | + | + | 6.5 |
| | 390 | 0 | | 0 | 5.0 |

* Infection.

there were 7 infected kidneys in 5 animals. None of the control animals was infected.

*Pathology of $NH_4Cl$-induced infections*

In order to carry out conclusive bacteriological studies, portions of experimental kidneys were not routinely examined histologically. Since, however, gross abscesses were found frequently, histological sections were prepared from hemisections of these kidneys, the remainder being taken for bacteriological study. The gross lesions were always wedge-shaped streaks of suppuration involving both medulla and cortex. Examples of such lesions are shown in Figures 3 and 4. They resemble those produced by direct inoculation of bacteria into the renal medulla.[5] The non-infected portions of the kidneys appeared normal.

Infection was never observed grossly in other regions of the body such as lung, liver, spleen, or peritoneum. Samples of lung, liver, and spleen cultured during the course of these studies were never found to be infected. Blood cultures were occasionally found to be positive in both experimental and control animals.

## DISCUSSION

The preceding experiments have demonstrated that measures designed to increase the activity of the renal ammonia-forming mechanism in the rat bring about an increased susceptibility of the kidney to infection. The effect seemed limited to the kidney and was independent of weight loss. The action of acidifying agents seemed to be chemical, since structural changes in the kidney could not be detected; furthermore, the increased susceptibility was promptly overcome by administration of an alkalinizing agent.

It is often difficult to measure the significance of increased tissue susceptibility to infection since, as is well known, the virulence of the particular bacteria employed is an important factor. For this reason it is important to note that the *E. coli* used in these experiments has not produced pyelonephritis in normal rats,[8] rats acutely depleted of potassium,[2] or rats poisoned with mercuric chloride.[18] This organism has also failed to cause pyelonephritis in rats with grossly visible nephrocalcinosis due to Vitamin D intoxication (400,000 units/d x 4).[6] It is thus not likely that the effect of $NH_4Cl$ was mediated by the increased urinary loss of K and Ca known to occur during acidosis since experiments designed to test the effect of these specific factors were negative.

The gross appearance of the lesions produced during ingestion of acidifying agents is similar to those resulting from direct inoculation of bacteria

FIG. 3. Hemisection of kidney of rat drinking $NH_4Cl$ seven days after intravenous inoculation of *E. coli*. Pale raised streaks of infection occupy more than half of this plane. Each division of ruler equals 1 mm.

FIG. 4. Surface of kidney shown in Figure 3 showing many abscesses beneath the capsule.

into the renal medulla. It is reasonable to presume that the medulla was the initial site of bacterial multiplication in the present experiments since the number of organisms required to produce infection there is much smaller than the number needed to initiate infection in the cortex.[8] Furthermore, unpublished observations from our laboratory have demonstrated that *E. coli* persisting in the normal kidney following intravenous injection are located almost exclusively in the medulla.[11] These findings point to local factors favorable to bacterial survival and multiplication in the renal medulla. The fact that both micropuncture studies[17] and stop-flow techniques[18] indicate that ammonia enters the urine mainly at the level of the collecting tubule is consistent with the thesis that local complement-inactivation may be one of the factors responsible for this peculiar zone of vulnerability. Since ammonia is highly diffusible, and since under certain conditions an elevated level of ammonia is demonstrable in renal vein blood,[10] it seems reasonable to postulate a comparatively high concentration in the interstitial tissues of the medulla, as well as in the tubular fluid.

The role of complement in natural resistance is not established with any certainty. Although lysis of many Gram-negative bacilli by the combination of complement and antibody is easily demonstrable *in vitro*, it must be kept in mind that some Gram-negative bacilli, including the strain of *E. coli* used in these studies, and all Gram-positive cocci, are resistant to lysis. It is entirely possible, nevertheless, that complement may have an effect on bacteria short of lysis which retards their rate of multiplication or renders them otherwise more susceptible to host defense. There is evidence that complement acts as an opsonin.[14] In a situation such as the one under study here only a slight tipping of the balance between host and parasite would be sufficient to change the result from no infection to infection.

The present experiments do not provide an explanation for the markedly increased renal susceptibility to infection attendant upon ureteral obstruction. Although it has been shown that there is immediate acidification of urine excreted during ureteral obstruction,[19] measurements of glutaminase activity of such kidneys revealed levels that were far below those which in the present experiments were associated with demonstrably increased susceptibility to infection.[7]

## SUMMARY

Using *E. coli* and *staphylococcus albus* as test organisms, administration of ammonium chloride or methionine was found to increase the susceptibility of rats to bacterial pyelonephritis.

Susceptibility tended to become greater after several days of acid ingestion. The effect was promptly overcome by alkali administration.

There was no evidence that the altered susceptibility caused by acidifying agents was related to structural alteration of the kidney.

Other tissues did not exhibit increased vulnerability to infection during the period of acid loading.

The findings are consistent with the suggestion that ammonia, formed in the kidney in response to acid stimulus, facilitates local growth of bacteria by interfering with complement activity.

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## BOOK REVIEWS

NEUROPHARMACOLOGY. H. A. Abramson, Ed. New York, Josiah Macy, Jr. Foundation, 1960. 251 pp. $6.00.

This volume comprises the edited transactions of the fifth conference on Neuropharmacology sponsored by the Josiah Macy Foundation and held in May 1959. This conference engaged in discussions on four main topics: (*i*) Amine metabolism and pharmacological implications; (*ii*) central action of chlorpromazine and reserpine; (*iii*) effect of serotonin on evoked potentials and; (*iv*) biochemical sites of action of psychotropic drugs. The value of these published proceedings lies more in bringing investigators in the field up-to-date on new developments and thinking in the area, than in providing background information for the student or uninitiated worker in the field. Moreover, this value is, as usual, diminished in proportion to the time which elapses between the actual conference and publishing of the proceedings (in this case, the time-lag has been about 18 months), and to the number of times similar material has appeared in other symposia and reviews.

The greatest proportion of this book is devoted to the topic of amine metabolism which is ably presented by Dr. S. Udenfriend, a competent and productive investigator in this field. In addition to a great deal of provocative and interesting information, Dr. Udenfriend raises a number of points which are not entirely clear to the reviewer. He implies, for example, that the localization of a neurohumor is an indication of where the substance is used (p. 12). While this is largely true, there are instances where it may be quite misleading: e.g. the large amounts of acetylcholine and serotonin in the spleen. Such accumulations might as well be indicative of regions in which enzymatic breakdown of the neurohumor is slow or non-existent. Another implication made by Dr. Udenfriend concerns the precursor of serotonin, 5-hydroxytryptophan (5-HTP). He states (p. 14) that as fast as 5-HTP is formed, it is decarboxylated. There is no question that the decarboxylase acts with high velocity, but it is possible that 5-HTP may be stored, as it is formed, a possibility which has not yet been systematically investigated. Raised also in this section by Dr. Udenfriend is the controversy over whether 5-HTP decarboxylase (which forms serotonin) and DOPA decarboxylase (which forms nor-adrenaline) are one and the same enzyme. Discussions in the future may be spared any more time devoted to this subject, since evidence has accumulated strongly in support of a single enzyme.

Dr. B. B. Brodie, another outstanding investigator in this field, presented some interesting ideas and facts about binding and release of serotonin. One weakness displayed by workers in this area, however, has been their willingness to accept platelets as model systems for studying phenomena of uptake and release. Platelets represent merely means of transportation for the amines, and in the reviewer's laboratory, for instance, it has been found that brain cells differ markedly from platelets in their characteristics of amine uptake. Another point made by Dr. Brodie to which this reviewer takes exception is his statement that iproniazid does not affect the release of

serotonin in platelets or other organs. Giarman and Schanberg (Biochem. Pharmacol. 1:301, 1959) showed that iproniazid does indeed hinder reserpine-induced release of serotonin from storage granules in rat's brain. Recently, the same has been found to be true about release of catecholamines from rabbit's atria.

These objections and criticisms are raised here only to present one of the apparent weaknesses of such conferences. Often, leading investigators in these discussions make incompletely substantiated assertions which are accepted as well-established facts.

Dr. K. Killam once again has presented in the second section of this volume his wife's and his work on an electrophysiological interpretation of the mode of action of the tranquilizers. They marshall strong evidence for establishing the reticular formation as a site of action of both reserpine and chlorpromazine. Especially attractive is their demonstration that both tranquilizers have the same action on this brain region: that of augmenting the effect of peripheral stimulation (input) upon it.

Dr. W. P. Koella has made use of a technique similar in some respects to that of Dr. Marazzi and co-workers, to arrive at a similar conclusion—that serotonin is an inhibitory neurohumor. Dr. Koella used the cortical evoked potential from a light flash on the retina. The most valuable part of his presentation is the analysis of his data to show that three factors are involved in this neuro-inhibitory action of serotonin: (*i*) stimulation of the carotid sinus; (*ii*) subcortical inhibition which has a bilateral effect; (*iii*) cortical inhibition whch is ipsilateral.

The last section of this book is devoted to another presentation of Dr. Brodie's hypothesis that Hess' central ergotropic system is adrenergic in characteristics of transmission, while his central trophotropic system is "serotonergic." This is, at best, a considerable over-simplification which challenges experimentation to refute it.

NICHOLAS J. GIARMAN

TEXTBOOK OF PHYSIOLOGY. By William D. Zoethout and W. W. Tuttle. 13th Edition. St. Louis, The C. V. Mosby Company, 1958. 712 pp. $5.25.

This volume is a well-written example of an intermediate level text of Physiology. It is aimed at those students desiring something less than the full treatment accorded medical students but, nevertheless, want material beyond elementary Biology.

The form of the text is conventional with a format found in many previous and present texts. The exposition starts at the very beginning with the first chapter on *Protoplasm and Life Processes* and proceeds rapidly through elementary Histology, Physical Chemistry, Biochemistry, the various organ systems, and ends with an adequate glossary of terms. The text is clear with frequent etymological breakdown of key words, meaningful to the Physiologist, but which may prove a stumbling-stone to the student, a relative neophyte.

I would recommend this text as a useful one to the serious Biology student. It might also prove to be helpful to medical students as a rapid review or to provide basic information missed in their undergraduate training.

In addition to the above recommendations, this volume has a function that may be valuable in the future. There is a tendency in some modern medical Physiology texts to start a study of excitation, for example, with an involved discussion of the "sodium hypothesis" and forgo all training in the phenomenology of the basic excitatory process. Thus, the student is unaware of the basic terminology and experiments that demonstrate excitation. At best this results in a lack of historical perspective on the part of the student. At worst, it leaves a tremendous gap in general knowledge.

If this trend continues, it may become necessary for medical students to buy a book of this type as an introduction to Physiology. However, until such a time is reached, this book will provide the needed foundation for more elaborate progression in Physiology at the Medical or Graduate School level.

JOHN T. CONRAD

CHEMOBIODYNAMICS AND DRUG DESIGN. By F. W. Schueler. New York, Toronto, London; McGraw Hill Book Company, 1960, 638 pp., $19.50.

Chemobiodynamics, according to the author, "represents the concept of chemical control as it manifests itself in an orderly way through action on the molecular level of the fabric of living things upward and onward, level by level, through the hierarchy of organized processes that are the warp and woof of chemical, cellular, organismal, and interorganismal being." This definition is typical of the author's style of writing. The book is richly larded with topics such as "topological cytochemistry as a key to the relationship between the polymolecular-system level and the cellular level of integration," "the aggregation of polymolecular systems in the orderly formation of cytoplasmic structures in functional transit," "the bucket-brigade principle (BBP)," or "pharmacopathology and the balance of nature."

There is no question that there is a real need for a book which would aid in bridging the gaps (now narrowing) between physicists, chemists, biochemists, pharmacologists, and physicians. However, the book being reviewed, while aimed at alleviating this need, is not successful in doing so.

While many topics are discussed, few are discussed adequately. Some of the author's ideas, such as his enthusiastic endorsement of mixed drug therapy, are controversial, others are interesting.

The book is inconsistent in the degree of knowledge expected from the reader. For instance, a rather lengthy discussion of the Schrödinger equation and quantum numbers is followed by a very brief description of "chemical bonds" in terms suitable for a high school text. Occasionally complex equations are shown without derivation or even definition of the terms used. Often rather simple concepts are made to appear complex, the author being

fond of statements such as "buffer action is ultimately dependent on a space-time frame of reference."

The main fault of this book, however, is that so much of it is outdated. It seems surprising that a text concerned with rational drug design should lack a discussion of protein structure and does not list deoxyribonucleic and ribonucleic acids in its index. Sulfonamide action is discussed without any mention of folic acid. The structures of coenzyme A and muscarine shown were proven to be incorrect several years ago. A discussion of acetylcholinesterase omits any mention either of "nerve gases," which act by profound inhibition of this enzyme, or of the compounds designed to reverse this inhibition. Analgesics are discussed in terms of "mixed sympathominetic and parasympathomimetic pharmacophoric moieties"; there is no mention of Beckett's concept of "analgesic receptor sites."

Factual errors, misprints, and incorrect structural formulae are numerous. Heterocyclical structures are often printed in a way which makes it difficult to see what compound is being depicted.

In view of the shortcomings and the high cost of this text it can be recommended neither to the physicians nor to the chemists for whom it was intended.

HENRY MAUTNER

STAINING METHODS. HISTOLOGIC AND HISTOCHEMICAL. By J. F. A. McManus and R. W. Mowry. New York, P. B. Hoeber, 1960. 423 pp. $10.00.

This is the sixth book of the same general nature that I have had the opportunity to review in the past two years. I, like the market, feel glutted. The book is of no particular worth to laboratories established in the field or individuals who investigate the criteria of the methods they use. For those beginning in the field or those merely seeking out a task-oriented technique, it has some value. The book is arranged in a rather thoughtful manner; that is, divided into five sections that are systematically organized. These sections are: (*i*) direct examination of tissue and preparation of tissue sections and smears; (*ii*) methods of general utility for the routine study of tissues; (*iii*) general methods for the study of the cell and its structures; (*iv*) special methods for the constituents of cell and tissues; (*v*) an interesting section of special recommendation concerning the study of special cells, tissues and organs.

The book contains several weaknesses, the most prominent of which are: (*i*) lack of photomicrographic illustration as an indication of model results with a method, (*ii*) inadequate bibliography, (*iii*) lack of discussion of criteria and validity of methods which leads to (*iv*) a somewhat weak and sometimes incorrect sections on the histochemistry of proteins and of enzymes. However, the intent of this volume is to provide a handbook integrating some of the newer methods of tissue examination into the standard laboratory procedures. It has succeeded.

RUSSELL J. BARRNETT

TROPHOBLAST AND ITS TUMORS. By William B. Ober (*et al.*) Annals of the New York Academy of Sciences, vol. 80, art. 1, pp. 1-284. New York, 1959. $3.50.

This series of papers is the result of a conference on "Trophoblast and its Tumors" held under the auspices of the New York Academy of Sciences in October 1958. It can be highly recommended, not only to Obstetricians and research workers in this field, but also to those not primarily interested in the problems of pregnancy. The book, together with its companion volume, "The Uterus," presents in a readable and collected form, authoritative accounts concerning important aspects of placental and uterine function. The sixteen papers in "Trophoblast and its Tumors" are divided into three sections, dealing in turn with Fundamental Concepts, Hydatidiform Mole and Trophoblastic Tumors. Two interesting basic aspects of trophoblast function also receive treatment in the third section, in two papers, one by W. W. Park and the other by W. A. Bardawil and B. L. Toy. Both discuss the problems of immunity which arise from consideration of viviparous pregnancy as a classical homograft situation. In addition, the latter authors devote considerable attention to another topic of current interest, the deportation of trophoblast in the blood stream, and give a comprehensive bibliography.

It is difficult to single out individual papers for special praise. All are of great interest and represent statements by leaders of their respective fields. In the section on Fundamental Concepts are included an historical survey, discussions of mechanisms of trophoblast invasion, and a commentary by J. T. Velardo on the evolution of viviparity and the role of the placenta and chorionic gonadotropin in the adoption of ovarian and pituitary function during pregnancy in the primate, based on the experimental actions of chorionic gonadotropin.

The second and third parts deal with pathological and clinical problems. The difficulties and importance of pathological classification are stressed and discussed by J. Smalbraak in relation to hydatidiform mole. This problem is considered in greater detail in Smalbraak's monograph and in the A.F.I.P. fascicle by A. T. Hertig and H. Mansell.

Dr. Delfs discusses her experience from following 129 patients with hydatidiform mole and choriocarcinoma by serum chorionic gonadotrophin determinations and she substantiates the value of this quantitative investigation which is of considerable interest to the practicing Obstetrician faced with such a problem.

It had been noted by a number of workers that the incidence of hydatidiform mole and choriocarcinoma was higher in certain parts of Asia than in the West. Lalla Iverson presents the results of a joint project for study of choriocarcinoma and hydatidiform mole in Asia. Hospital figures from reliable clinics in Asia indicate an incidence of hydatidiform mole as high as 1:200 to 1:250 deliveries while that of choriocarcinoma ranges from 1:250 to 1:2708 deliveries, with the true figure probably lying in between. For comparison, Hertig and Mansell give incidences of 1:12,500 to 1:40,000 pregnancies for chorioadenoma destruens and choriocarcinoma respectively.

Although it is stressed by the authors that at best these figures are an informed estimate, and also taking into account the change in denominators, it is nevertheless apparent that a considerable difference exists. Iverson, reporting the finding of the joint project, presents convincing data, with statistical analysis, and discusses sampling problems and possible etiologic factors.

Choriocarcinoma occurs in men, both in the testis and in extragenital sites. These tumors are of great interest for a number of reasons but two deserve special comment. The first is the puzzling observation of a higher incidence of such tumors in men, compared with similar tumors, of non-trophoblast origin, in women. The second is the possible significance of the finding that approximately one-half of the choriocarcinomas and teratomas in men possess the Barr nuclear chromatin particle, or "female" marker. These and other problems are discussed in the interesting review by N. B. Friedman.

The final paper presents the encouraging results obtained by Roy Hertz and his group in the treatment of choriocarcinoma and related trophoblastic tumors in women with the folic acid antagonist, "methotrexate." The rationale of this mode of therapy is based on the high folic acid requirement of tissues of both the fetus and the maternal genital tract.

Again it is regrettable that the Annals of the New York Academy of Sciences do not contain subject indexes which, it is felt by this reviewer, would contribute appreciably to the usefulness of such publications, especially to the casual reader. The organizers of the conference are to be congratulated for arranging this varied program with an international flavor which includes contributions of a uniformly high standard in nearly all fields of current interest in trophoblast.

MICHAEL GALTON

DICTIONARY OF WORD ROOTS AND COMBINING FORMS. By Donald J. Borror. Palo Alto, California, National Press Publications. 1960. 134 pp. $2.00.

The key to understanding most scientific terms is their "derivation," the meanings of the Latin and Greek roots from which these words were built. Professor Borror offers a compact and useful list of such roots and their translations in his new *Dictionary*. Also included in the pocket size, flexible cover volume is a helpful introduction and lists of combining forms. A helpful little book.

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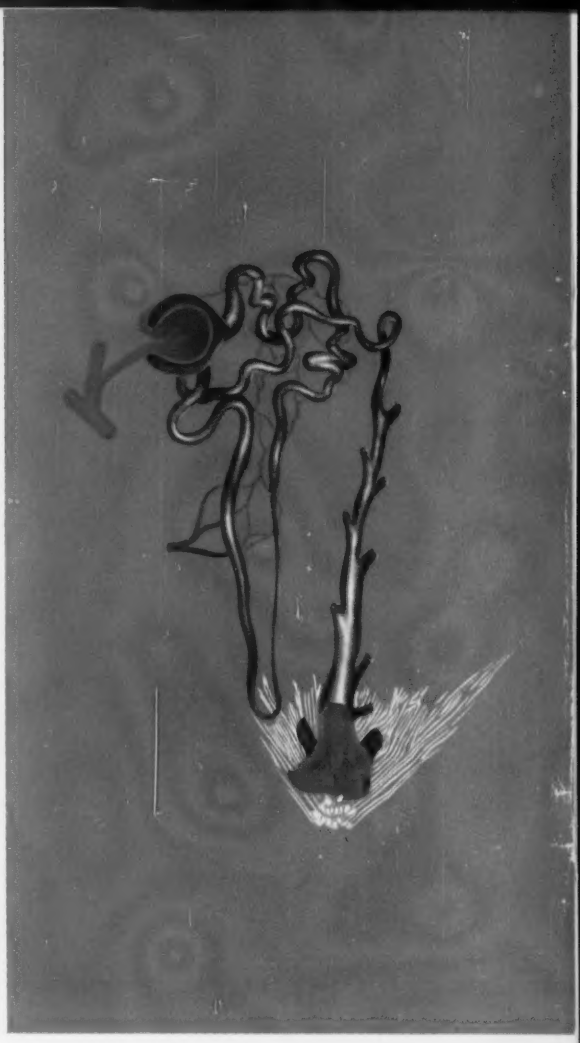

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References: 1. Schreiner, G. E.: A.M.A. Arch. Int. M. **102**:32, 1958. 2. Freedman, L. R., and Beeson, P. B.: Yale J. Biol. & Med. **30**:406, 1958. 3. Rocha, H., et al.: Yale J. Biol. & Med. **30**:341, 1958.

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